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VOLUME V OCTOBER, 1912 PART I

STUDIES ON SOIL PHYSICS.

PART II.—THE PERMEABILITY OF AN IDEAL SOIL TO AIR AND WATER.

BY HEBER GREEN AND G. A. AMPT.

Introduction.

§ 1. In Part I. (vol. IV. pp. 1—24) it was shown that the intrinsic permeability of a soil when measured with water was less than when measured with air as the experimental fluid, and that the ratio of the two values varied with the amount of colloidal matter present. It was desirable to determine accurately whether the values would be identical for soils composed of pure sand or other uniform and non-colloidal particles.

It should also be possible to calculate the permeability of a soil from a knowledge of the sizes of its component particles and of the specific pore-space; and this problem is one that has engaged the attention of several authors both from the experimental and mathematical standpoints.

Previous Investigations.

§ 2. Allen Hazen (*Ann. Rep. State Board of Health*, Mass., U.S.A., 1892), in an investigation of filter-bed sands and gravels, used an experimental filter filled with various grades of sands, and deduced the formula

$$v = cd^2 \frac{h}{l}(0{\cdot}70 + 0{\cdot}03\theta),$$

where c is a constant, approximately 1000; d is the effective diameter of sand grain; h is the head of water pressure; l is the length of sand column; θ is the temperature; and v is the velocity of water-flow in metres daily.

§ 3. King and Slichter (*Nineteenth Annual Report*, 1899, Part 2, of the U.S. Geol. Survey), in a very complete and masterly research, have not only experimentally measured the permeability of sands, soils and rocks to both air and water, but the latter has developed a mathematical solution of the problem.

Slichter's mathematical argument may be summarized as follows:—

If we consider a soil composed of spherical particles, then the lines joining any eight contiguous spheres will be found to outline a parallelopiped varying in form from a cube to a rhombohedron, according as the particles are packed in the loosest or closest manner. This unit element of volume with length of side d will contain the equivalent of a single sphere of diameter d.

"If the grains of soil are arranged in the most compact manner possible, each grain will touch surrounding grains at twelve points, and the elements of volume will be a rhombohedron having face angles equal to 60° and 120°." "If the grains are not arranged in the most compact manner, the rhombohedron will have its face angles greater than 60° and each sphere will touch other spheres in but six points and *nearly* touch in six other points." "The most open arrangement of the soil grains, which is possible with the grains in contact, is had when the rhombohedron is a cube."

For a rhombohedron whose side is of unit length the volume is given by $(1-\cos\theta)(\sqrt{1+2\cos\theta})$, where θ is the face angle; and consequently the pore-space S, not occupied by the enclosed sphere, is given by

$$S = 1 - \frac{\frac{\pi}{6}}{(1-\cos\theta)\sqrt{1+2\cos\theta}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1).$$

The values of S for different values of θ are thus readily calculable and are given in Table I.

Slichter finds, that if l be the length of the soil column under consideration and d the diameter of each particle, then the length of the pore-space capillary may be taken as equal to $\frac{l(1-\cos\theta)}{\sin\theta\sqrt{1+2\cos\theta}}$, and the area of its minimum cross-section as equal to

$$\frac{\sin\theta - \frac{\pi}{4}}{2} \cdot d^2.$$

A mathematical and experimental investigation showed that the error involved in assuming the pore to be *circular and equal in area*

to the minimum cross-section of the actually triangular pore is almost balanced (to within one per cent.) by the assumption that the pore is straight instead of curved.

Hence, using the same nomenclature as in Part I. (*loc. cit.* § 6), we may write Poiseuille's equation

$$\frac{v}{t} = \frac{\pi}{8\eta} \cdot \frac{ghs}{l} \cdot \Sigma r^4 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

in the form

$$\frac{v}{t} = \frac{\pi}{8\eta} \cdot \frac{ghs \sin\theta \sqrt{1+2\cos\theta}}{l(1+\cos\theta)} \cdot \frac{\left(\sin\theta - \frac{\pi}{4}\right)^2}{4\pi^2} \cdot d^4$$

$$= \frac{ghsd^4}{32\eta l} \cdot \frac{\sin\theta\sqrt{1+2\cos\theta}}{1+\cos\theta} \cdot \left(\sin\theta - \frac{\pi}{4}\right)^2 \ldots\ldots\ldots\ldots\ldots(3),$$

for each of the two pores penetrating a unit element, *i.e.* the rate of flow per area $\frac{\sin\theta}{2} \cdot d^2$ which is half the area of cross-section occupied by each rhombohedron.

Then if the cylinder containing the column of soil be of area A,

$$\frac{v}{t} \text{ (for the whole area)} = \frac{ghsAd^2}{16\pi\eta l} \cdot \frac{\sqrt{1+2\cos\theta}}{1+\cos\theta} \cdot \left(\sin\theta - \frac{\pi}{4}\right)^2$$

$$= \frac{ghsAd^2}{16\pi\eta l} \cdot \frac{(1-\cos\theta)(\sqrt{1+2\cos\theta})\left(\sin\theta - \frac{\pi}{4}\right)^2}{\sin^2\theta} \quad \ldots\ldots\ldots(4).$$

But from equation (1)

$$1 - S = \frac{\frac{\pi}{6}}{(1-\cos\theta)(\sqrt{1+2\cos\theta})};$$

therefore

$$\frac{v}{t} = \frac{ghsAd^2}{96\eta l} \cdot \frac{\left(\sin\theta - \frac{\pi}{4}\right)^2}{(1-S)\sin^2\theta}$$

$$= \frac{ghsAd^2}{96\eta l} \cdot \frac{\left(1 - \frac{\pi}{4}\operatorname{cosec}\theta\right)^2}{1-S} \ldots\ldots\ldots\ldots\ldots(5).$$

Put $B = 1 - \frac{\pi}{4}\operatorname{cosec}\theta$, and take $g = 980$ and $s = 1$, then

$$\eta \cdot \frac{v}{th} \cdot \frac{l}{A} = 10{\cdot}2d^2 \cdot \frac{B^2}{1-S} \quad \ldots\ldots\ldots\ldots\ldots(6),$$

and putting $$k = \frac{1-S}{B^2},$$

we get $$\eta P = 10{\cdot}2\,\frac{d^2}{k} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7).$$

The values of S and the mathematically derived constants B and k were calculated by Slichter for various values of θ and are given in Table I. It will be seen that k varies from 11·37 for the loosest packing to 85·43 for the most compact arrangement possible with perfect spheres.

TABLE I.

Values of the "permeability constant" (k), as calculated by Slichter, for all possible variations of pore-space (S) when dealing with a soil composed of spherical particles.

S Pore-space	θ Angle of packing	B $=1-\frac{\pi}{4}\operatorname{cosec}\theta$	k $=\frac{1-S}{B}$
·2595	60° 00′	·0934	85·43
·30	62° 36′	·1155	52·45
·31	63° 18′	·1210	47·1
·32	64° 3′	·1266	42·45
·33	64° 49′	·1322	38·45
·34	65° 37′	·1378	34·75
·35	66° 27′	·1434	31·6
·36	67° 21′	·1491	28·8
·37	68° 18′	·1549	26·26
·38	69° 17′	·1605	24·08
·39	70° 20′	·1661	22·1
·40	71° 28′	·1719	20·3
·41	72° 43′	·1775	18·75
·42	74° 3′	·1832	17·3
·43	75° 32′	·1890	15·95
·44	77° 10′	·1946	14·75
·45	79° 6′	·2003	13·70
·46	81° 25′	·2057	12·75
·47	84° 59′	·2117	11·83
·4764	90° 00′	·2146	11·37

§ 4. The desirability of testing the formula

$$\eta P = 10{\cdot}2\,\frac{d^2}{k}$$

experimentally for both air and water is self-evident and King has carried out, in conjunction with Slichter, an elaborate series of measurements with sands of various sized grains.

A summary of the results obtained in one series [*Fifteenth Annual Report*, Agr. Expt. Stn. Univ. Wisconsin, p. 127] is given in Table II.

TABLE II.

Summary of experiments, by F. H. King, on the Permeability of Sands to Air and Water.

Measured diameter of sand grains	Permeability to water calculated from			Average percentage error
	known diameter of grains	observed flow of air	observed flow of water	
	$P_w = c\frac{d^2}{\eta_w k}$	$P_w = P_a\frac{\eta_w}{\eta_a}$	$P_w = \frac{v}{t}$	
2·755	2680	2277	2296	− 17·5
1·993	1372	1132	1080	− 24
1·588	909·1	757	756	− 20
1·345	638·6	522	542	− 20
1·157	499·6	453·2	504·6	− 4·5
1·106	326·6	297·5	329·2	− 4
·802	194·0	193·0	210·0	+ 3·5
·665	106·2	122·0	138·6	+22·5
·582	75·7	80·6	94·8	+16
·489	59·8	66·8	72·3	+16

They confirm the formula to within a little more than experimental error, but it must be borne in mind that King's apparatus allowed a very considerable margin for this factor. King himself admits these discrepancies and goes on to say,—"Search has been made thus far in vain for some medium consisting of spherical grains of perfectly uniform diameter, with which to secure a rigid test of the method, but, as yet, none has been found."

Shot are unsuitable, partly on account of their weight, but also because the finest dust shot the present authors can obtain have a diameter of about 1 mm. and much finer sizes are required.

Preparation of Material.

§ 5. *A suitable material has however been found* in the "glistening dew" of the picture post-card artist. This is composed of almost perfectly spherical grains or beads of glass ranging in diameter from 0·25 mm. upwards. Enquiries have been made for "beads" of still smaller sizes, but such are apparently not manufactured; if obtainable

they would be extremely valuable for an extension of this and similar researches on soil physics and the phenomena of adsorption.

Two varieties were used; the smaller of which (0·5 mm. or less) were composed of colourless glass, often surface stained with aniline dyes, whilst the larger (up to 1 mm.) were coloured with various metallic oxides.

Sifting was soon found to be a tedious method of grading them, for the sieves became almost immediately choked and were with difficulty freed from beads which were but little larger than the perforations.

§ 6. Finally an elutriation method was devised, based on the ordinary processes used for the mechanical analysis of soils.

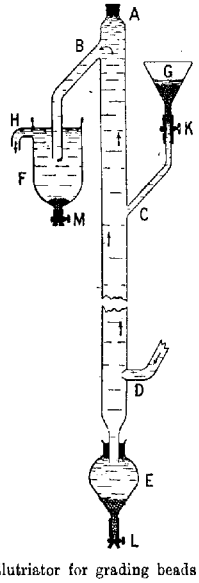


FIG. 1. Elutriator for grading beads and sands.

The apparatus[1] consisted of a vertical tube *ABCDE* about six feet long and one inch in diameter, provided with an inlet tube at *D* with a constricted jet for the water supplied from a constant level reservoir.

The water passed upwards and out at *B* to the cylinder *F*, finally escaping to the waste by overflowing at *H*. After they have been

[1] We are indebted to Mr Radcliff of the Bairnsdale School of Mines for suggesting this shape of elutriating tube.

cleaned by successive treatment with caustic soda and aqua regia, the beads to be graded are poured into the funnel *G* and flow in a stream, regulated by the clip *K*, through *C* into the main elutriation tube where they meet the upflowing current of water.

Those beads having a diameter greater than the "critical diameter" for the water current will sink past *D* into *E* and can be drawn off through *L* as required: the lighter beads will rise and be carried over into *F* where they will settle and may be removed by opening the clip *M*.

Many precautions were found necessary, as Hilgard has pointed out for his soil elutriator, and even under the best conditions it is impossible to obtain a perfect separation. When many beads are present in the tube *BC* it will no longer have the same efficient area as when empty of beads, and so the velocity of upward flow of the water becomes materially increased and beads having more than the critical diameter will be carried over into *F*. Another cause of error is the variation in stream velocity with the distance from the walls of the tube and often beads may be observed travelling upwards for several inches in mid-stream, and then, after approaching the wall, tumbling downwards again.

Occasionally beads have hovered about in the tube for two or three days, but even these on microscopical examination exhibit a considerable variation in their diameters.

§ 7. The separations obtained were the results of many repetitions of this elutriation process, care being taken to put each sample through slowly. The original mixture was thus classified into thirteen grades, of which five were selected for the final experimentation.

§ 8. The average diameter of the beads in each grade was determined by counting at least two thousand beads and taking weighings at regular intervals. As each bead was separately picked out with a small pair of forceps and transferred to a weighing bottle the process became very tedious, especially in the case of the smaller sizes. In all, some 40,000 beads were counted out in this way—one by one.

The density of each grade was determined by displacement of water in a specific gravity bottle—it was found to vary considerably with the size. The two larger sizes being coloured with metallic oxides showed a corresponding increase of density.

The density of these beads was found to be 3·117 and their average diameter was therefore 0·9374 mm.

The discrepancy between the weights of the separate lots of beads

was greater than would have been expected either from the method employed in sorting them or from their appearance under the microscope (see Pl. I, figs. 8 and 9). The discrepancy was less in the case of the smaller beads but greater for the sand grains used in the later part of this work. The large numbers counted and weighed must however have eliminated any perceptible error due to this cause.

TABLE III.

Weight of large coloured glass beads.

Weight of first thousand	=1·3319	Weight of sixth thousand	=1·3861
,, second ,,	=1·3946	,, seventh ,,	=1·3149
,, third ,,	=1·3428	,, eighth ,,	=1·2835
,, fourth ,,	=1·3467	,, ninth ,,	=1·4070
,, fifth ,,	=1·3027		

Average weight per thousand=1·3456

Standardization of Apparatus for measuring Permeability.

§ 9. As the tubes filled with beads had a very much larger permeability than the soils previously dealt with, the rates of flow for both air and water no longer conformed to Poiseuille's simple capillary tube law[1] and were sufficiently high to make the correction for loss of kinetic energy of the moving fluid an appreciable factor.

In order to determine the value of this and other corrections, and to test the accuracy of the method, the special apparatus employed was first used to measure the permeability of a straight capillary tube whose constants could be exactly obtained from its dimensions.

This capillary tube was carefully calibrated with a thread of clean mercury: the variations in diameter amounted to less than one per cent.

Length of capillary = 54·90 cm.

Average area of cross-section = 0·00745 sq. cms.

Then using the same nomenclature as in Part I. of this paper [*vide* this *Journal*, vol. IV. pt I. pp. 3—6], from Poiseuille's equation

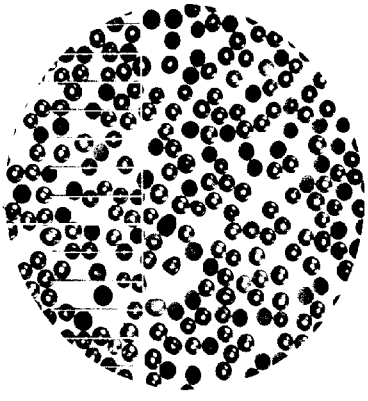
$$\eta = \frac{r^4 \pi g}{8} \cdot \frac{hst}{lv},$$

and taking

$$P = \frac{v}{t} \cdot \frac{l}{hs},$$

$$\eta P = \frac{\pi g}{8} \cdot r^4.$$

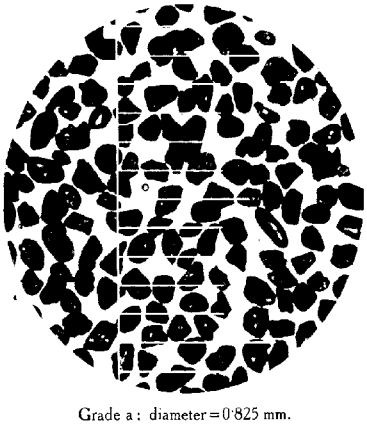
[1] See Vol. IV. p. 3, § 6.



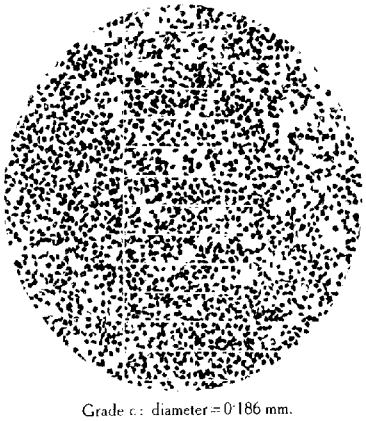
Grade B: diameter = 0·709 mm.

Grade E: diameter = 0·250 mm.

Fig. 8.



Grade a: diameter = 0·825 mm.



Grade c: diameter = 0·186 mm.

Fig. 9.

Therefore, for the tube in question,

$$\eta P = 0{\cdot}002153.$$

§ 10. *Permeability to air.* The corrections applied were:—

(i) *For loss of kinetic energy.* The well-known Couette-Finkener formula is

$$\eta = \frac{\pi}{8} \cdot \frac{ghst}{vl} \cdot r^4 - \frac{\rho}{8\pi} \cdot \frac{v}{lt},$$

where ρ is the density of the fluid (air); then as

$$P_{\text{obs.}} = \frac{vl}{th},$$

$$P_{\text{corr.}} = P_{\text{obs.}} \left(1 + \frac{\rho}{8\pi} \cdot \frac{v}{\eta lt}\right)$$

$$= P_{\text{obs.}} \left\{1 + \frac{\rho P_{\text{obs.}}^2}{\pi^2 l^2 g r^4} . h\right\},$$

which for the capillary tube in question simplifies to

$$P_{\text{corr.}} = P_{\text{obs.}} (1 + 0{\cdot}00107h).$$

(ii) *For compressibility of the air.* Meyer, Breitenbach and others have shown that Poiseuille's law when applied to gases should take the form

$$\eta = \frac{\pi}{8} \cdot \frac{r^4 t}{vl} \cdot \frac{p_1^2 - p_2^2}{2p_0},$$

where p_0 is the pressure under which the air is measured;

p_1 „ „ of air at the *high* pressure end of the capillary;

p_2 „ „ „ „ *low* „ „ „ „

As pressures were measured in centimetres of water in the gauge, this correction reduced to

$$v_{\text{corr.}} = v_{\text{obs.}} \left(1 + \frac{2h_0 - h}{2B + h}\right)$$

for experiments in which a "head" of pressure was employed, and to

$$v_{\text{corr.}} = v_{\text{obs.}} \left(1 + \frac{h - 2h_f}{2B - h}\right)$$

for experiments in which a "tail" of pressure was employed, *i.e.* where the pressure in the bulb C was less than atmospheric.

B = barometric pressure expressed in cm. of water;
h = mean working pressure;
h_0 = initial pressure in measuring bulb;
h_f = final pressure in measuring bulb.

(iii) Corrections were also applied for the *"dead space"* in the connecting tubes between the measuring bulb and the capillary tube, and for the slight alteration in volume due to movement of the water in the pressure gauge.

All these corrections were summarized for our apparatus in the following equation, which, though complicated in appearance, was found to be simple in application:

$$P_{\text{corr.}} = P_{\text{obs.}}(1+0{\cdot}00107h)\left[1+\frac{2h_0-h}{2B+h} \text{ or } 1+\frac{h-2h_f}{2B-h}\right]$$
$$\left\{1+\frac{1}{v}\left(\frac{h_0-h_f}{20}+\frac{h_0-h_f}{14}\right)\right\}.$$

§ 11. The apparatus, shown in Fig. 2, consisted of a measuring bulb C, of known capacity between the marks B and D, connected on

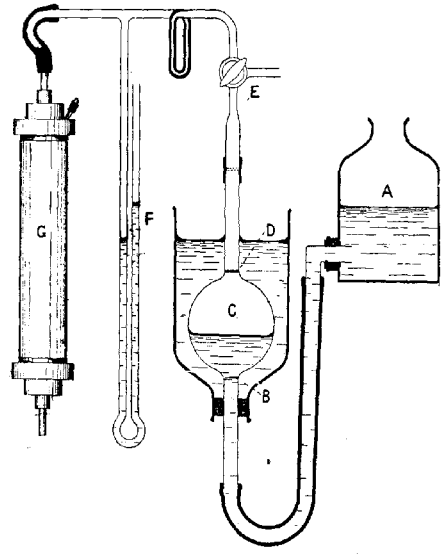


FIG. 2. Apparatus used for the measurement of the permeability to air of a glass tube filled with beads.

the one side to an adjustable water reservoir A, and on the other to the tube G containing the glass beads (or the capillary tube) whose permeability to air was to be measured.

To carry out an experiment the reservoir A was adjusted so as to give the required pressure and, by means of the three-way tap E, air was either forced or aspirated through until the water level was several centimetres either below B or above D according as the measurements were to be made under "head" or "tail" of pressure. The tap E was then turned so as to make communication between C and G, and the water level rapidly rose as the air in C was being forced through G. At the moment of passing B the stopwatch was started and the first reading of the pressure at F obtained by means of a reading telescope; further readings were made at regular time intervals and the watch stopped as the water level passed D.

The mean pressure h was obtained by averaging these readings, half weight only being given to the initial and final pressures (h_0 and h_f).

Whilst the temperature coefficient for the viscosity of air is only small, yet it is necessary to waterjacket the bulb C to prevent *changes* of temperature, and therefore of effective volume, *during* the progress of an experiment. For a similar reason C and G must be maintained at the *same* temperature; consequently, when the capillary tube was being experimented on, it was enclosed in a Liebig's condenser through which the water that had circulated round C was passed.

TABLE IV.

Time readings	h (cm.) (*a*) using "head" of pressure	h (cm.) (*b*) using "tail" of pressure	
			Temp. = 9·7° C.
Initial	9·75	10·7	
15″	7·4	8·1	(*a*) Using "head" of pressure;
30″	6·7	7·45	t = 3·425 mins.
45″	6·1	6·8	h = 4·53 cm.
1′ 0″	5·6	6·2	
1′ 15″	5·15	5·7	
1′ 30″	4·75	5·25	(*b*) Using "tail" of pressure;
1′ 45″	4·35	4·75	t = 2·855 mins.
2′ 0″	3·95	4·3	h = 5·41 cm.
2′ 15″	3·55	3·85	
2′ 30″	3·25	3·35	
2′ 45″	2·85	2·8	
3′ 0″	2·4	—	
3′ 15″	2·0	—	
Final	1·05	1·9	
Mean value of h	4·53	5·41	

A typical set of readings are given in Table IV for a pair of experiments in which a "head" and "tail" of pressure respectively were used.

§ 12. A number of experiments were made with this capillary tube under widely different conditions of pressure in order to decide whether the formulae given by Meyer and others would hold good for our apparatus and eliminate the various errors due to loss of kinetic energy etc.

TABLE V.

Measurements of the Permeability (to air) of a glass capillary tube.

t (secs.)	h (cms.)	$P_{obs.}$	h_0	h_f	$P_{corr.}$	Temp.	$\eta \times 10^6$	$\eta P_{corr.}$
			(i) *Using "head" of pressure*					
54·8	+17·84	11·64	23·4	14·5	12·10	8·8°	177·2	·002144
67·0	14·47	11·74	19·9	11·0	12·13	8·8°	177·2	2148
83·1	11·57	11·85	17·0	8·15	12·19	10·0°	177·8	2168
100·6	9·44	11·96	14·8	5·95	12·26	9·0°	177·2	2172
125·7	7·54	12·00	12·95	4·05	12·27	9·0°	177·2	2174
171·9	5·47	12·09	10·9	2·0	12·31	9·4°	177·4	2184
215·5	4·35	12·22	9·85	1·05	12·36	9·4°	177·4	2192
250·4	3·67	12·38	9·25	0·5	12·38	9·8°	177·7	2236
			(ii) *Using "tail" of pressure*					
50·3	−19·28	11·83	24·8	16·0	12·06	10·5°	178·0	·002146
53·5	17·89	11·87	23·35	14·5	12·10	9·7°	177·7	2152
58·9	16·24	11·88	21·6	12·75	12·12	9·9°	177·7	2152
67·1	14·18	11·93	19·5	10·7	12·13	10·1°	177·8	2154
69·3	13·61	12·03	18·95	10·1	12·23	9·6°	177·6	2176
80·3	11·83	11·95	17·0	8·2	12·13	10·1°	177·8	2154
96·3	9·86	11·97	15·0	6·1	12·15	9·6°	177·6	2156
117·6	8·015	12·06	13·05	4·3	12·22	9·6°	177·6	2170
140·0	6·71	12·10	11·8	3·05	12·26	9·7°	177·7	2178
171·3	5·41	12·28	10·7	1·9	12·43	9·7°	177·7	2208
205·4	4·53	12·23	9·75	1·05	12·36	9·7°	177·7	2196

§ 13. Both of these series of experiments (in Table V) exhibit a slight regular progression, and seem to indicate that the various corrections leave some residual effect unbalanced. It was found difficult to eliminate the error due to differing surface tension forces acting on the water in the two limbs of the manometer; but the zero error, sometimes 0·1 or 0·2 mm., was always read and allowed for.

The true value of ηP should apparently be obtained by working at an infinitely small pressure or by extrapolation of the values obtained

at higher pressures, but owing to the shape of the measuring bulb the lowest mean pressure that could be worked with, in the manner described above, was about 4 cms. The device was therefore adopted of inserting a "resistance in the circuit," between E and G, in the shape of a suitable piece of capillary tubing. This allowed of mean pressures of less than 1 cm. being utilized without loss of accuracy although initial and final readings of the pressure in the bulb (h_0 and h_f) as well as at the manometer (h_1 and h_2) had to be taken in order to apply the usual corrections indicated in § 10.

TABLE VI.

Measurements of the Permeability (to air) of a glass capillary tube, using a "resistance" capillary in the circuit.

t (secs.)	h (cms.)	$P_{obs.}$	h_0	h_f	h_1	h_2	Temp.	$\eta P_{corr.}$
216·9	−4·299	12·23	23·5	14·7	5·37	3·41	10·0°	·002166
216·7	−4·276	12·28	23·5	14·7	5·37	3·41	8·5°	2164
240·9	+3·960	11·90	22·1	13·3	5·16	3·09	10·0°	2174
240·9	+3·960	11·92	22·1	13·3	5·16	3·09	9·0°	2170
603·7	+1·579	12·15	12·0	13·1	2·82	0·73	9·8°	2194
607·5	−1·512	12·35	11·5	2·6	2·69	0·64	9·4°	2198
990·7	−0·940	12·20	9·3	0·4	2·20	0·13	10·2°	2195
1053·5	−0·887	12·17	9·5	0·6	2·18	0·10	10·2°	2192

§ 14. All the values of $\eta P_{corr.}$ obtained are shown in Fig. 3 and it will be seen that the "gang" is independent of experimental error and that the results for both "head" and "tail" pressures, with and without

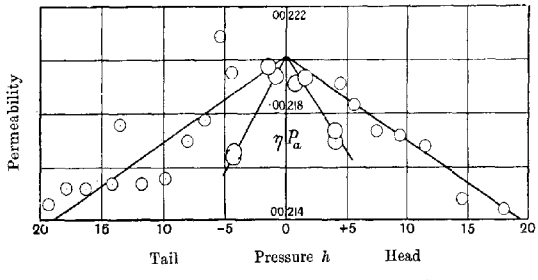


FIG. 3. Permeability of capillary tube to air under varying pressures.

the capillary in series, all extrapolate almost exactly to $\eta P = 0{\cdot}002202$. This is 2·2 per cent. greater than 0·002153 the value calculated from

the measured dimensions of the capillary and taking $\eta_0 = 0{\cdot}0001730$ [Meyer and Breitenbach]; but it must be borne in mind that the absolute value for the viscosity of air obtained by different observers varies by at least this amount.

Consequently we may fairly claim for this apparatus an accuracy quite sufficient for our needs.

§ 15. *Permeability to water.* The water was supplied from a large reservoir and after passing through the capillary was, in the preliminary experiments, allowed to drip from the curved exit tube into the receiving vessel—the head of pressure being taken as the difference in level between the orifice of this exit tube and the water in the reservoir.

The capillarity effects at the outlet are considerable however, and were overcome by reading the pressure from two side tubes connected to each end of the tube whose permeability is being measured. The nozzle of the outlet tube had also to be immersed in liquid and placed in contact with the wall of the burette that was used as a receiving vessel, otherwise a fluctuation of pressure was observed as each drop detached itself from the orifice.

The results obtained in a series of experiments at different pressures are given in Table VII. The decrease of ηP_w at low pressures is to be accounted for by the loss due to evaporation of the effluent water, and the percentage error due to this cause will evidently be proportional to the reciprocal of the rate of flow or, more conveniently, driving pressure.

TABLE VII.

Measurement of the Permeability (to water) of a glass capillary tube.

h (cms.)	$\eta_w P_w$	$\frac{1}{h}$
19·39	0·002158	·052
11·21	2148	·089
10·41	2147	·096
10·20	2143	·098
4·119	2107	·243
3·025	2087	·331
2·048	2028	·489
1·980	2030	·506
1·098	1907	·911
0·56	1740	1·787

The results obtained for $\eta_w P_w$ are therefore plotted (in Fig. 4) against $1/h$ and the graph is, as was expected, a straight line extrapolating to $\eta P = 0{\cdot}002173$.

The kinetic energy correction, even for the highest rates of flow, is almost insignificant and only raises this extrapolated value of ηP to 0·002183.

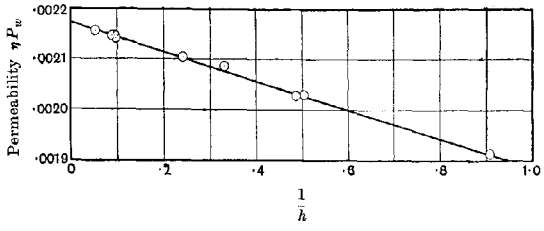


FIG. 4. Permeability of capillary tube to water under varying pressures.

§ 16. All the results obtained with this capillary tube may be summarized thus:

ηP (calculated from the measurements of the tube) = 0·002153,
$\eta_a P_a$ (from observed permeability to air) = 0·002202,
$\eta_w P_w$ (from observed permeability to water) = 0·002183.

The accuracy shown here is abundantly sufficient for the purpose in view.

Construction and Testing of Apparatus for holding Beads.

§ 17. Many measurements were made of the permeability of each of the various grades of beads when contained in narrow tubes, such as had been previously used for soils, but it was found that the error in the measurement of the pore-space in these narrow tubes was too great for the results to be of value in our present enquiry.

§ 18. A special containing vessel, shown in Figs. 2 and 5, was therefore designed. It consisted of a glass tube with brass cone-shaped end pieces which were carefully ground to fit the glass tube and were capable of exact measurement before being cemented in place. The inner surfaces of these brass ends were turned into hollow cones whose apices were connected with the exterior by means of short pieces of brass tubing into which corks and glass tubes were inserted.

The beads were retained in place by a disc of wire gauze (shown in the figure by a line of dots) and were fed in through a small hole drilled diagonally through the brass ends. This arrangement allowed

of the accurate determination of volume and other dimensions and proved thoroughly satisfactory.

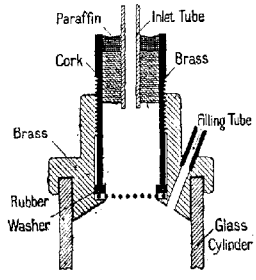


FIG. 5.

A knowledge of the volume is necessary to determine the pore-space S, and $\frac{l}{A}$ is also required since it enters into the formula

$$P = \frac{v}{th} \cdot \frac{l}{A}.$$

The value of l/A was separately determined for the body of the tube and the conical ends.

For a truncated cone

$$\frac{l}{A} = \frac{1}{\pi \tan \alpha}\left(\frac{1}{r} - \frac{1}{R}\right)$$
$$= \frac{h}{\pi r R},$$

where α is the angle of the cone, r and R the radii of its top and base respectively and h its length.

§ 19. The measurements of the cones and tubes, made with specially accurate calipers, were as follows for the two vessels that were employed.

Vessel I was only used in the earlier experiments as the glass cylinder was repeatedly breaking. Nearly all the recorded experiments were made with Vessel II which was carefully made to fit a glass tube of better quality and very [illegible]m bore.

§ 20. Bead sample (diameter = 0·319 mm.; density = 2·750) was first experimented with. Vessel I was loosely filled with about 200 grams of the beads and its permeability to air measured by means of the apparatus described in § 11; then, by gentle packing, an extra one or two grams of beads were introduced and the permeability again measured. This

process was repeated until, even on continued "dumping" and rotating, the tube would hold no more beads.

TABLE VIII.

Dimensions of Vessels in which Permeabilities of Beads were measured.

	Vessel I		Vessel II	
Area of cross-section of glass cylinder...	7·09 cm.²		3·567 cm.²	
Length of glass cylinder.....	12·13 cm.		23·60 cm.	
Length of cones = h	0·394	0·362	0·328	0·342
Radius of base of cones = R	1·473	1·470	1·052	1·048
Radius of apex of cones = r	0·720	0·720	0·500	0·482
Volume of cones	1·55 cm.³	1·42 cm.³	0·65 cm.³	0·66 cm.³
Value of l/A for cones.....	0·119	0·109	0·198	0·215
Total volume of vessel	88·97 cm.³		85·50 cm.³	
l/A for whole vessel.....	1·943		7·03	

After measuring the permeability to air for this minimum pore-space, the tube was connected with a water-pump and exhausted. Air-free distilled water was allowed to enter and remain at rest for some considerable time to dissolve any air that had escaped removal by pumping.

The permeability to water was then measured by observing the rate of flow through the column under a measured pressure of water.

TABLE IX.

Preliminary measurements of the Permeability of Bead Sample D in an unlined tube.

S	$k=\frac{1-S}{B^2}$	$\eta_a P_a \times 10^3$	$\eta_w P_w \times 10^3$	$\frac{\eta_a P_a}{d^2} . k$
0·379	26·06	0·780		19·97
0·3695	26·39	·746		19·34
0·366	27·28	·7075		18·95
0·364	27·79	·682	0·515	18·62

§ 21. The disagreement between $\eta_a P_a$ and $\eta_w P_w$ in the last line of Table IX is too great to be accounted for by ordinary experimental

errors and was eventually found to be due to imperfect removal of the air from between the interstices of the beads. In subsequent experiments special precautions were taken to ensure the complete removal of the air and the disagreement practically vanished.

More difficult of explanation however were the values of $\frac{\eta_a P_a}{d^2} . k$ given in the last column; these should, from Slichter's calculation (see § 3), be equal to 10·2. The high permeability observed might, it was thought, be due to the channels, of a larger area than those between the beads themselves, existing all round the inner surface of the tube (Fig. 6). The error due to this cause will depend on the relative diameters of the beads and the containing tube. Where the diameter of a bead is one per cent. of that of the tube a simple calculation shows that the permeability will be increased by about twenty-eight per cent., if the beads are packed in the closest possible manner; for ordinary packing the error will be diminished to about eight per cent.

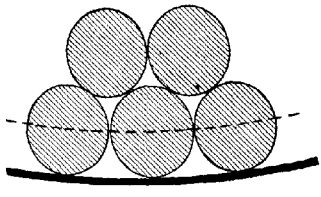


FIG. 6.

But the want of concordance with Slichter's formula cannot be accounted for in this way.

§ 22. If the wall of the tube could be covered with hemispheres fitting closely together, this correction would be practically eliminated; this condition was attained by the following indirect device.

The inner surface was lined with a layer of cement (Chatterton's compound) approximately calculated to be sufficient to embed the beads to half their diameter. In order to obtain an even coating the cement was dissolved in chloroform and poured into the vessel and the chloroform completely evaporated by blowing air through the tube while the latter was continuously rotated in a horizontal position. The tube was then filled with beads and gently warmed to melt the cement into which a layer of beads sank and was firmly held on cooling.

The cement and half of the embedded beads were considered as belonging to the wall and the other half of the glass beads to the main

body whose permeability and pore-space were to be measured. In calculating the latter, half the weight of these embedded beads was added to the weight of the loose beads with which the vessel was filled.

The "effective" volume and area of the tube were obtained from appropriate weighings of the attached cement and beads. In one case the following measurements were obtained:

Unlined tube: original volume = 89·0 c.c.; $\frac{l}{A} = 1{\cdot}943$.

The inner surface was then lined with cement (cones excepted). 5·80 grams of beads—diameter = 0·319 mm., density = 2·75—attached themselves and occupied a volume of 2·10 c.c.

Lined tube: volume (determined by water content)	= 85·15 c.c.
half-volume of beads in lining	= 1·05 c.c.
"effective" volume	86·20 c.c.
volume of cones (from previous measurement)	= 2·97 c.c.
„ cylinder (by difference)	= 83·23 c.c.
length „	= 12·04 cm.
therefore area of lined part of cylinder	= 6·910 sq. cm.
and $\frac{l}{A}$ for „ „ „	= 1·744
but $\frac{l}{A}$ for cones (from previous measurement)	= ·228
therefore "effective" $\frac{l}{A}$ for whole vessel	= 1·972

§ 23. Table X gives the results of the first experiments carried out with the tube lined as described above; and in Fig. 7 curves are drawn comparing the unlined and lined tubes filled with the same beads.

TABLE X.

Measurements of the Permeability of Bead Sample D in a lined tube.

S	$k = \frac{1-S}{\beta^2}$	$\eta_a P_a \times 10^3$	$\eta_w P_w \times 10^3$	$\frac{\eta_a P_a}{d^2} \cdot k$
0·3895	22·19	0·901		19·63
0·3835	23·39	·851		19·55
0·3835 (?)	23·39	·811		18·62 (?)
0·373	25·62	·736		18·51
0·3625	28·17	·626	0·611	17·31

Whilst attempting to duplicate these measurements the glass tube broke and it was at this stage that the second vessel was constructed and brought into use. It was also lined with cement and a series of readings obtained with it, for the same size of beads is included in Table XI and in Fig. 7.

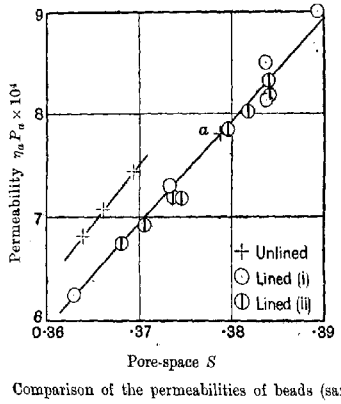


FIG. 7. Comparison of the permeabilities of beads (sample *D*) in unlined and lined tubes.

It will be seen that while the two lined vessels give results agreeing within about one per cent., the beads in the unlined tube had a permeability some seven per cent. higher. This agrees with the estimate previously given (§ 21).

The highest pore-space which we attempted to work with in the unlined tube gave a point, marked (*a*), considerably below the true curve; obviously the slight vibration due to unavoidable handling of the apparatus further compacted the beads and the reading must therefore be discarded. With the lined tubes there was naturally much less tendency for this to occur.

Results obtained with Glass Beads.

§ 24. Series of measurements were now made with five sizes of the carefully graded glass beads, the glass tube being in each case lined with some of the same size beads embedded in cement. After the permeability to air was observed for several degrees of compactness, then water was admitted as described and a reading of the permeability to water obtained.

TABLE XI.

Summary of the Permeabilities of Glass Beads, of various grades, as measured in lined tubes.

Diameter of "beads"	Pore-space	Intrinsic Permeability		$\frac{\eta P}{d^2} \times 10^3$	$\frac{\eta P}{d^2} \cdot k$
		to air	to water		
d	S	$\eta_a P_a \times 10^3$	$\eta_w P_w \times 10^3$		
A 0·938 mm.	0·391	6·76		7·68	17·10
	·370	5·21		5·92	15·54
	·3675	5·125		5·82	15·65
	·3635	4·96	4·70	5·63	15·69
				5·33	14·90
B 0·709 mm.	0·400	4·685		9·31	18·90
	·3925	4·19		8·33	18·02
	·388	3·93		7·80	17·52
	·384	3·806		7·56	17·61
	·373	3·264	3·33	6·48	16·58
				6·63	16·93
C 0·497 mm.	0·376	1·776		7·18	17·89
	·373	1·677		6·77	17·33
	·366	1·538		6·22	16·96
	·366	1·564		6·34	17·28
	·361	1·489	1·523	6·02	17·18
				6·15	17·57
D 0·319 mm.	0·3895	0·901		8·85	19·63
	·3835	·851		8·37	18·62
	·373	·736		7·22	18·51
	·3625	·626	0·611	6·15	17·31
				6·00	16·90
	0·384	0·831		8·15	18·98
	·3795	·785		7·71	18·65
	·3745	·719		7·06	17·88
	·3705	·692	0·6615	6·795	17·77
				6·52	17·05
	0·382	0·802		7·875	18·65
	·3735	·7195		7·065	18·00
	·368	·676	0·655	6·63	17·73
				6·43	17·40
E 0·250 mm.	0·3905	0·516		8·25	18·30
	·384	·470		7·525	17·52
	·379	·4385		7·02	17·05
	·373	·4135		6·62	16·94
	·370	·4035		6·45	16·94
	·370	·410		6·56	17·03
	·366	·377	0·373	6·03	16·45
				5·97	16·27

§ 25. The summarized results are given in Table XI and Fig. 10 and a satisfactory basis for discussing the accuracy of Slichter's calculations for spherical particles of uniform size within the range of the diameters available. The values of k used throughout this paper are those taken from his formulae (see Table I).

Results obtained with Sands.

§ 26. It was decided to extend the investigation to ordinary sand grains also of uniform size. The quartz sand utilized was but slightly water-worn and, after being chemically cleaned, three grades were separated by sifting and elutriation by the same apparatus and in the same manner as the glass beads.

The accompanying photographs enable their shape, size and uniformity to be examined and compared. The smaller grades originally contained much ilmenite, the bulk of which however was removed by careful "panning off."

§ 27. The average diameter of each grade was determined in the usual way by counting out several thousand grains and weighing them. Much greater deviations from the mean were found than with the glass spherical beads, possibly due in part to the variations in composition and consequently of specific gravity.

The actual weighings were as follow:—

TABLE XII.

Diameter and Specific Gravity of Sand Grains.

	Coarse Sand		Medium Sand	Fine Sand
Number weighed	1000		500	500
Weighings	0·7364	0·7251	0·0170	0·0045
	·7261	·7883		·0045
	·6933	·9207	·0167	·0045
	·7105	·8226		·0043
	·7415	·9076		·0045
Average weight per thousand ...	0·7802 gram.		0·0337 gram.	0·00892 gram.
Specific Gravity	2·654		2·653	2·648
Average Diameter	0·825 mm.		0·289 mm.	0·186 mm.

§ 28. The same apparatus was used for measuring the permeabilities of the quartz sands to both air and water as had previously been used

for the glass beads. It was considered unnecessary to line the vessel with cement and a layer of the sand on account of the irregular and angular shapes of the individual grains. The results justified this decision.

TABLE XIII.

Summary of Permeabilities of Quartz Sands.

Diameter of Grains	Pore-space	Intrinsic Permeability		$\frac{\eta P}{d^2} \times 10^3$	$\frac{\eta P}{d^2} \cdot k$
		to air	to water		
d	S	$\eta_a P_a \times 10^3$	$\eta_w P_w \times 10^3$		
a 0·825 mm.	0·386	3·186		4·68	10·71
	·378	3·018		4·43	10·84
	·3695	2·706		3·975	10·48
	·361	2·515		3·71	10·68
	·3485	2·144		3·155	10·12
	·347	2·070		3·04	9·89
			2·026	2·97	9·68
b 0·289 mm.	0·412	0·447		5·35	9·87
	·393	·3640		4·36	9·40
	·385	·3265		3·91	9·03
	·381	·3065		3·67	8·76
	·378	·2955		3·535	8·66
	0·408	0·4525		5·42	10·33
	·395	·383		4·585	10·08
	0·403	0·4005		4·795	9·51
	·385	·3385		4·055	9·36
	0·401	0·3885		4·65	9·37
	·373	·294		3·525	9·02
			0·294	3·525	9·02
c 0·186 mm.	0·435	0·2525		7·30	11·20
	·4045	·1820		5·265	10·33
	·3945	·1650		4·78	10·17
	·377	·1320		3·82	9·44
			0·1290	3·74	9·22

Discussion of results.

§ 29. The justifiability of Slichter's assumptions and calculations may be judged by the agreement of these results with the formula

$$\eta P = 10{\cdot}2 \frac{d^2}{k}.$$

I.e. the expression $\frac{\eta P k}{d^2}$ should be equal to 10·2 for all sizes of particles and for each arrangement of pore-space.

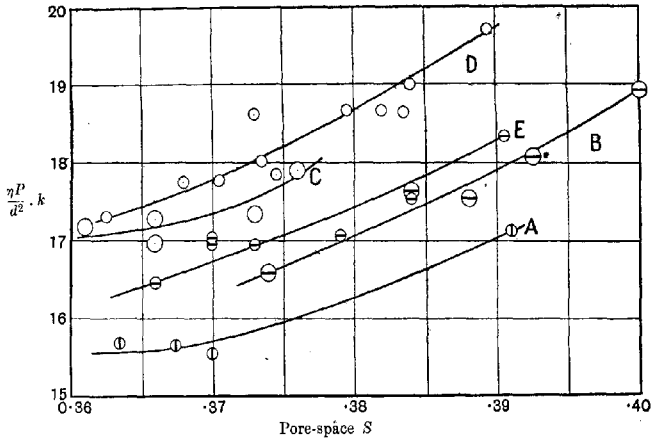


FIG. 10. Summary of results with glass beads (see Table XI).

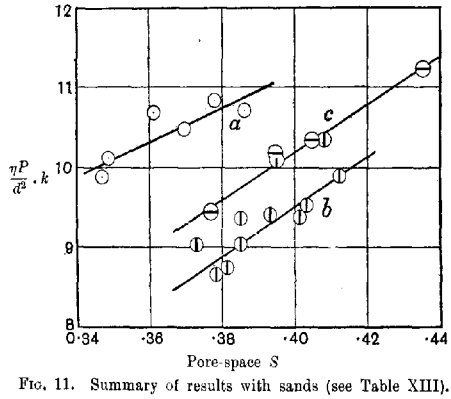


FIG. 11. Summary of results with sands (see Table XIII).

§ 30. It will be observed that for the glass beads however no such agreement is to be found, for the value obtained varies from 15·5 to 19, and increases with increase of pore-space. In other words, the permeabilities both to air and water are from 50 to 85 per cent. greater than Slichter's calculated values.

The explanation of this is almost certainly to be found in his method of considering each soil capillary as if it were a double triangular-shaped pore with a *partition down the centre* instead of as an *undivided* more or less rhomboidal pore at its narrowest part. It is obvious that this assumption must considerably undervalue the permeability of the pore,

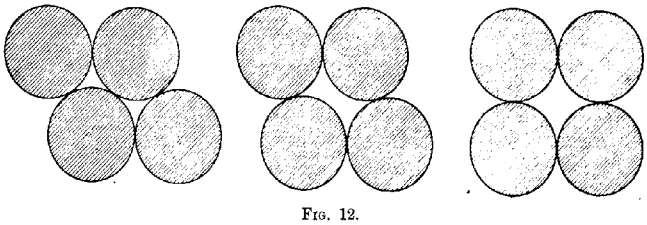


FIG. 12.

but it would be difficult to make anything like an exact estimate of the deficiency. Any such correction should vanish when the angle of packing is sixty degrees, for, as the pore-space approaches the minimum possible, so does the pore actually become divided into two and Slichter's method of discussion would then hold good. [See Fig. 12.]

An examination of the graph (Fig. 10) bears this out, for if extrapolated to the minimum pore-space the value of $\frac{\eta P k}{d^2}$ evidently will in general approximate to the calculated figure—10·2.

§ 31. On the other hand, the experiments with ordinary sands, though showing a rather large percentage error, give an average result of 9·45 for the permeability to air ($\eta_a P_a$) and 9·31 for the permeability to water ($\eta_w P_w$). This is, considering the difficulties of accurate measurement, a satisfactory concordance.

The obvious explanation (of this less perfect material agreeing more perfectly with the theoretical formula) is that the angular shapes of the particles do practically have the effect of dividing the pore into two triangular passages as assumed in the formula.

Conclusion.

§ 32. As the particles in ordinary soils are not perfect spheres but more or less angular in shape, *the experiments described in this paper show that the formula* $\eta P = 10{\cdot}2\,\frac{d^2}{k}$ *holds quantitatively for variations of the pore-space and of the diameters of the soil particles.* This will be so

whether the permeating fluid be *air or water, provided that the actual sizes of the soil particles are unaffected by the presence of water*[1].

With this factor taken into account it is therefore legitimate to consider a soil as statistically composed of a bundle of capillary tubes when discussing the movements of air and water through it.

In conclusion we have to acknowledge our indebtedness to Professor T. R. Lyle for valuable advice and suggestions, to Mr H. J. Grayson for assistance in preparing the micro-photographs and to the Victorian Government for financial assistance towards the expenses of this research.

[1] It was pointed out in Part I. of these researches (*loc. cit.* §§ 9, 32) that the behaviour with water is a most important property of the soil; for whereas with clean sands the ratio $\frac{\eta_a P_a}{\eta_w P_w}$ will be but slightly greater than unity, the amount of colloidal matter present will cause a corresponding increase in its magnitude.

INVESTIGATIONS ON "SICKNESS" IN SOIL.

I. Sewage Sickness.

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The experiments of Russell and Hutchinson[1] have shown that the micro-organic population of ordinary soils is not working at a maximum efficiency; there exists a biological factor, provisionally identified with the soil protozoa, detrimental to bacteria and limiting their numbers and activities. It follows that any change in the conditions of the soil that is more favourable to the harmful organisms than to the bacteria will disturb the normal equilibrium between these two sets of organisms and lead to a relative reduction in bacterial numbers and activity. We shall therefore expect to find that causes not in themselves harmful to bacteria may bring about a reduction of bacteria through favouring the development of the detrimental factor.

About three years ago the soil of the Kegworth sewage farm, in which one of us is interested, showed marked signs of "sewage sickness." When the sewage was run on to the land it no longer percolated as rapidly as before or gave rise to so pure an effluent: instead it lay about in pools and the effluent was unsatisfactory. It was no longer possible to apply sewage while the crops were growing as is done on a larger farm not far away: when this was attempted with mangolds or cabbage the leaves began to fall and the plants were finally killed. On going over the ground together and taking careful note of the phenomena it seemed that we had here a case where the

[1] This *Journal*, 1909, **3**, 111.

state of affairs mentioned above is realised, and the bacterial efficiency was being kept down by excess of a detrimental factor; we therefore decided to investigate the problem from this point of view. The Kegworth Parish Council kindly gave us the necessary permission, allowed us access to the farm at all times, and granted us facilities for carrying out our experiments, for which we desire to tender to them our very sincere thanks.

The land treatment of sewage consists in allowing the sewage to run on to the land with or without a preliminary treatment. At Kegworth the sewage is simply screened and then applied to land that has been ploughed and is later to be cropped; elsewhere it is applied direct to growing crops. So long as percolation is rapid the method works well, but it breaks down, and "sewage sickness" sets in, directly percolation becomes too slow. So real is the danger that only particular types of soil are suitable for sewage farming, a light loam lying on a sand being perhaps one of the best[1]. Indeed the chief point in the management of a sewage farm seems to be to arrange the drainage system, the distribution of the sewage and the cropping in such a manner as to prevent the land from becoming waterlogged. Thus at the Aldershot camp sewage farm, where, Col. Jones[2] informs us, sewage sickness is unknown, the sewage is applied direct to rye grass immediately after a crop has been taken and is run on till the land is sufficiently wet or till the crop has grown too tall, when no more is applied till another crop is cut. It is also run on to land carrying mangolds. The third crop, oats, however, receives no sewage. When we saw the crops their healthy condition was sufficient proof of the absence of waterlogging.

Examination of the sewage sick field showed that the falling off in the rate of percolation was due to at least two causes. Wherever a pool of sewage had stood we found a greenish-black slime, black below the water-level and green at and above it, which under the microscope was seen to contain living organisms—algae, *euglena* and numerous others. The black material also penetrated into the soil for a short distance. A second cause is the deflocculation of the clay by the free

[1] Such a soil may take 30,000 gallons of sewage per acre per day, equivalent to nearly $1\frac{1}{2}$ inches of rainfall. Other land, however, may take only 3000. At Birmingham the average quantity has been 6000 (Watson, J. D., *Proc. Instit. Civil Engineers*, 1910, CLXXXI, Part III).

[2] The details of Col. Jones' management are described in *Natural and Artificial Sewage Treatment*: Col. A. S. Jones and H. A. Roechling, London, 1902. The land treatment of sewage is also fully discussed in the *5th Report of the Sewage Commission*.

alkali of the sewage[1]. Both these causes render the soil sticky and impermeable. They can, however, be put out of action: the deflocculation by dressings of lime, and the accumulation of black slimy material by ploughing up the land into ridges and allowing it to dry. Both devices are successfully adopted in sewage farming.

But there is another factor involved both in the wet soil and in the drier and better aerated "rested" soil. Our experiments show that in no case is the bacterial efficiency as high as might be expected. Counts made by the gelatine plate method showed that some 20 to 30 millions of bacteria were commonly present per gram of wet sewage-sick soil, and no marked rise set in when the moisture was reduced and the air supply increased to what might have appeared more favourable proportions. Considering the large amount of easily decomposable nitrogenous organic matter present, and the enormous stimulus such compounds afford to the multiplication of bacteria, these numbers must be regarded as distinctly low; the poor arable soils investigated by Russell and Hutchinson contained 10 to 15 millions per gram, which numbers ran up to 150 millions per gram after addition of 0·1 per cent. of peptone. It thus appears that some factor is keeping down the bacterial population in a sewage-sick soil even under favourable aeration conditions.

This detrimental factor is, however, put out of action by treatment with small quantities of antiseptics (toluene and carbon disulphide were used in the experiments) and the bacterial numbers then rise rapidly to between one and four hundred millions per gram; a great increase in the rate of ammonia production also takes place (Tables II and III). We know of no ordinary soils where partial sterilisation produces anything like so marked an effect; it is clear that we are dealing with a detrimental factor of unusual potency.

It was soon found that the effect of partial sterilisation was an improvement in the soil as a medium for bacterial activity, and not an improvement in the bacterial flora. Indeed the new flora proved to be actually less vigorous than the old when both were placed under similar conditions; for example, it did not attain to such high numbers or bring about so much decomposition as the old flora introduced into the partially sterilised soils and therefore living in equally favourable

[1] In the present case the sewage is mainly domestic, there being no trade effluent except from one large brewery.

The question is more fully discussed by us in *Journ. Soc. Chem. Ind.* 1911, **30**, 471, "Sewage sickness in soil and its amelioration by partial sterilisation."

circumstances (Table V). Thus in one experiment the untreated soil contained 31 million bacteria per gram, the soil treated with carbon disulphide contained 110 millions per gram, but the same treated soil to which the original flora had been introduced by inoculation with 0·5 per cent. of the untreated soil contained 475 millions; now that the bacteria of the original soil have as good a chance of multiplication as those of the partially sterilised soil they increased to a markedly higher extent.

The unsuitability of the untreated "sick" soil for bacterial growth is not due to an accumulation of toxic substances by bacteria because there is no falling off in numbers on the partially sterilised soils even after three months in spite of their very high bacterial content, so long of course as the soil does not become too dry. Further, the very susceptible nitrifying organisms worked quite well in the untreated soil, and they would hardly have done so in presence of a toxin.

The addition of an aqueous extract of untreated sick soil to partially sterilised soil had no adverse effect on the growth of the bacteria or on the amount of decomposition (Table VI). The harmful factor is thus not carried by a water extract; it is neither a soluble toxin nor bacteria.

When, however, some of the untreated soil was added to the partially sterilised soil there was a large reduction in bacterial numbers, especially in the active forms, and the rate of decomposition fell off considerably. The reduction did not set in at once but took some weeks to manifest itself (Table VI). Thus the harmful factor is slowly transferred to the partially sterilised soil on contact with the untreated soil. It shows, in fact, the phenomena of growth and is therefore presumably biological in character.

These phenomena are in complete agreement on all points with those observed on normal soils, and the conclusion seems irresistible that the same detrimental factor comes into play in both cases, but its action is intensified in the sewage-sick soils. Reference to the paper by Russell and Hutchinson will show the reasons for considering the harmful factor in ordinary soils to consist in amoebae and other protozoan organisms. Our experiments with sewage-sick soils lead us to conclude (1) that a factor detrimental to bacteria occurs in sewage-sick soils and is precisely similar in nature to that present in ordinary soils, (2) the detrimental factor occurs to an abnormal extent in the sewage soil, and therefore appears to be much favoured by the mixture of water and organic matter that constitutes sewage, this being the condition that brings on sewage sickness, (3) one of the reasons for

sewage sickness is the reduced bacterial efficiency consequent on the abnormal development of this injurious factor. These conclusions, it will be observed, are statements of experimental facts and are independent of any hypothesis as to the nature of the injurious factor, but they accord extremely well with the protozoan hypothesis, which was based on a wholly different set of considerations.

The difficulty in the way of finding out precisely which are the harmful organisms in a normal soil is much increased when we pass on to sewage-sick soils. The flora and fauna are very extensive and have not been adequately explored, although it is clear that they would repay investigation from the very interesting work of Fowler[1] and of Meixner[2] on the effluents from bacterial beds. Some of the forms are no doubt helpful in the decomposition process, but it is a simple matter to demonstrate the presence of amoebae and other protozoa known to feed on bacteria, and even to separate living forms from the sewage soil by proper centrifuging.

Whichever the harmful organisms may be, they are much more susceptible to the effects of antiseptics than are bacterial spores, and are readily killed by toluene, carbon disulphide, or heat. When that has been done the bacteria, as we have seen, multiply more freely and effect a greater amount of decomposition, and the soil regains or more than regains its original efficiency as a purifying agent. A number of experimental filters made of sewage-sick soil were set up equal in every respect and equally dosed with sewage, but differing in that some were partially sterilised while others were not. In all cases the effluents from the partially sterilised filters were decidedly better than those from the untreated filters, indicating a higher degree of purification (Tables VII and VIII). This conclusion was confirmed by treating a large area of land with toluene, laying drains and comparing the drainage water with that from a similar but untreated area. Again it was found that the purification was more complete after partial sterilisation.

Thus it appears that no complicating factor comes into play when our laboratory results are applied to field conditions, and we may draw the practical conclusion that the biological factor detrimental to bacteria flourishes in sewage treated soils and reduces the efficiency of the soil

[1] Fowler, G. J., *City of Manchester Rivers Dept. Annual Report*, 1911. We understand that Dr Fowler and Mr Crabtree are extending these interesting observations.

[2] Meixner, A., "The fauna of the Bradford coke bed effluent" (*Proc. Camb. Phil. Soc.* 1908, **14**, 530).

as a purifying agent. The physical factors in sewage sickness may be overcome by methods already in use: the deflocculation by addition of lime or chalk, and the formation of slime on the surface by deep ploughing and exposing the land in high ridges to dry. Both methods are recognised features of good sewage farm practice. But neither of them kills the factor injurious to bacteria, for we found it to persist in our laboratory experiments where the texture of the soil, the aeration and the water supply were all that could be desired; indeed from our results we should expect to find it in all sewage farm soils. In order to kill this factor and to secure maximum bacterial efficiency it is necessary in addition partially to sterilise the soil either by heat or by antiseptics. Paring and burning proved to be an efficient method of heating the soil, but the application of antiseptics on the large scale presents difficulties that have not yet been overcome. Both toluene[1] and carbon disulphide can be obtained cheaply, but it is not necessary to restrict attention to these; any antiseptic may be expected to serve provided it is volatile or decomposes with formation of innocuous compounds when its work is done. Experiments with various other antiseptics are in hand in connexion with the partial sterilisation of horticultural soils, and there is every reason to suppose that any method suitable for these would also prove suitable for sewage-sick soils.

Experimental.

Analysis of the soil. The soil was taken from a field that had been receiving sewage for some months past and was about to lie up for a time. It was strongly alkaline to litmus paper and coated in places just above the little pools with a dark green slimy matter and in places where water had lain with a black deposit that penetrated a little way into the soil. Its composition on different dates is given in Table I.

In the wet winter samples the amount of ammonia is extraordinarily high in comparison with the 1 or 2 parts per million found in ordinary arable soils. In the drier May sample the ammonia is lower but the nitrates have now risen considerably.

The ammonia is estimated by distillation *in vacuo* with magnesia[2] and the "nitrate, etc." by extraction with water, boiling off all ammonia from the extract after addition of magnesia, acidifying with acetic acid, reducing with a zinc-copper couple and estimating the ammonia thus

[1] We are now paying 10*d.* a gallon for commercial "toluole."

[2] Russell, this *Journal*, 1910, **3**, 233.

formed. The qualification "etc." is added because we find that rich soils such as our sewage soil yield up to water other reducible and non-volatile compounds besides nitrates, so that the results always come out too high. For our present purpose, however, the total reducible nitrogen compounds give a better measure of the amount of decomposition than the nitrates alone.

TABLE I. *Analysis of the sewage-sick soil.*

	Nov. 20, 1910	Jan. 5, 1911	Feb. 10, 1911	May 24, 1911
Moisture present, per cent.	36·6	38·3	31·6	20·6
Loss on ignition, per cent.		12·7		
Composition of dry matter				
Carbonates (as $CaCO_3$), per cent.		0·6		
Total nitrogen, per cent.		0·41		
Nitrogen as ammonia, per million	180	150	100	24
,, ,, nitrate, per million	10	16	31	134
Bacteria present, millions per gram of moist soil		37[1]	21[1]	

In order to make the bacterial counts about 10 grams of soil were placed in a weighing-bottle and weighed, then tilted into a graduated litre flask containing about 750 c.c. of sterile distilled water. The flask was corked and shaken for half an hour, sterile water was added to the litre mark and the whole shaken again. 1 c.c. was then transferred to 100 c.c. sterile distilled water and the mixture was agitated for three or four minutes, 1 c.c. of this was transferred to another 100 c.c. sterile distilled water and shaken, finally 1 c.c. of this last mixture was added to the gelatine and poured on to the plates. Instead, however, of the ordinary Petri dish we used the special form one of us[2] had for some time adopted for bacteriological work. The method is not capable of great refinement and duplicate counts do not agree very closely, but the differences obtained in our experiments are far beyond the errors of the determination.

We have not studied the bacterial flora in any detail, but judging from the appearance of the colonies it did not appear to be very varied

[1] Owing to the very great difficulty of sampling, these numbers are necessarily only approximate.

[2] Golding, "A New Bottle for Cultures," *Journal of the Society of Chemical Industry*, 1906, **25**, p. 677.

in any of the soils. From the soil treated with carbon disulphide (Table II) we obtained numerous little matted colonies forming a firm pellicle difficult to break with a platinum needle: these on further examination were found to consist of long chains or threads of the leptothrix type not motile on gelatine but somewhat motile on agar. From the toluened soil (Table II) a number of thinnish grey colonies with darker centres were obtained, while the reinfected toluened soil (Table V) yielded in addition a number of smooth yellow colonies. The brown forms, some of which were threads, varied in their occurrence; more were found in the stored soil (Table III) than in any other samples; all were killed by heat and carbon disulphide, some, however, escaped the action of toluene in the Table III series but not in the rather different Table II series. There were remarkably few liquefying organisms in any of the samples.

Moulds developed freely in the heated soils but not in the heated and limed soils (Table III).

The effect of partial sterilisation on bacterial activity. The soil as it came to the laboratory was much too wet for experimental purposes; it was therefore allowed partially to dry, passed through a 3 mm. sieve and then divided up into a number of equal portions of 500 grams each. These were put into wide-mouthed bottles plugged with sterile cotton-wool and subjected to their appropriate treatment: some were heated to 98° C. for three hours, while others received 2 per cent. of carbon disulphide or toluene and after two days were spread out at ordinary temperature till the antiseptic had completely evaporated; water was then added to bring the soil to the proper state of moistness.

In the experiments recorded in Table II the soil was treated as soon as possible after it came from the farm. Dealing first with the January 5 sample: a comparison of the data for the untreated soil as it was freshly taken (Table I) with the results given here shows that the removal of the excess of water has remarkably little effect on the bacterial numbers or on the amount of decomposition. There is a considerable immediate loss of ammonia—from 150 parts per million in the wet soil to 70 parts in the drier soil—but very little change took place in the untreated soil after the ninth day in spite of the very favourable temperature, moisture and aeration conditions. Bacterial multiplication and the production of ammonia are both more rapid and carried to a further extent in the soil treated with carbon disulphide.

The most rapid decomposition, however, is in the heated soil, but

TABLE II. *Influence of partial sterilisation on bacterial activity in sewage-sick soils fresh from farm.*

(*a*) Sample taken January 5, 1911. Moisture reduced to 12 per cent.

	Bacteria present per gram of dry soil, millions			Nitrogen present as ammonia, parts per million of dry soil		Nitrogen present as nitrate, parts per million of dry soil		Sum of ammonia + nitrate, parts per million of dry soil	
	At start Jan. 5	After 9 days	After 22 days	After 9 days	After 22 days	After 9 days	After 22 days	After 9 days	After 22 days
treated soil	37[1]	31	31	74	91	12	9	86	100
ated to 98°		22	28	128	257	12	13	140	270
eated with CS_2 ...		130	110	93	139	16	8	109	147

[1] On wet soil, 41 per cent. of water being present.

(*b*) Sample taken May 24, 1911. Moisture reduced to 16 per cent.[2]

	Bacteria present per gram of dry soil, millions			Ammonia present, parts per million of dry soil			Nitrate present, parts per million of dry soil		
	After 2 days	After 15 days	After 4 months	After 2 days	After 15 days	After 4 months	After 2 days	After 15 days	After 4 months
treated soil	Plates	44	43	25	25	12	140	191	327
ated to 98°	spoiled	34	735	35	233	378	109	108	150
eated with toluol ...	by hot	222	272	76	210	277	84	85	157
	weather								

[2] At 16 per cent. of moisture the soil is in a very nice moist condition eminently suited for the growth of plants.

	Ammonia + nitrate present, parts per million of dry soil		
	After 2 days	After 15 days	After 4 months
Untreated soil	165	216	339
Heated to 98°	144	341	528
Treated with toluol ...	160	295	434

here the bacteria only multiply very slowly and the flora is greatly restricted, only very few species showing on the plates. This is the usual course of events on heated soils and indicates some fundamental difference between the effect of heat and of antiseptics that we have not yet investigated. We know, however, that a chemical change is brought about by the heat because the soil takes on a characteristic odour, yields a darker coloured aqueous extract and becomes specially favourable for the development of moulds and unfavourable for the development of the sensitive nitrifying organisms[1]. A change in physical properties is also brought about by heat.

The May samples show the same general relationships; the first bacterial counts were lost by the liquefaction of the plates during a sudden spell of very hot weather, but subsequent counts show very little change in numbers in the untreated soil; there is, however, a steady and continuous decomposition, which is clearly normal and unaffected by any circumstance more harmful to the sensitive nitrifying organisms than to the others because nitrification is proceeding more rapidly than ammonia production. Toluene treatment leads to a great rise in bacterial numbers and a considerable increase in the amount of decomposition. Heat, as before, causes the greatest increase in the rate of ammonia production but no increase in bacterial numbers in the early stages. Later on, however, the bacteria rise to an enormous extent, but the flora is still very restricted and there is a considerable development of mould.

The experiments recorded in Table III were designed to ascertain whether the harmful factor is removed by treatment with lime or by long exposure to air. The soil was partially dried to reduce the moisture to 16 per cent. and then kept for seven weeks (from June 23 to August 10, 1910) under very favourable conditions of aeration, moisture and temperature; it was then subdivided and treated as before. An additional set was introduced here in which the soil was heated to a temperature sufficiently high to cause a certain amount of charring. The results are of the same kind as when the soil is freshly treated and show that the injurious factor is not removed by aeration or by lime.

In this case initial bacterial counts were taken throughout, and they show as usual that partial sterilisation much reduces the numbers, heat being particularly drastic in its effects. In the soils treated with toluene and carbon disulphide the bacteria rapidly multiply to a marked

[1] See also Russell and Hutchinson, this *Journal*, 1909, **3**, 111.

TABLE III. *Influence of partial sterilisation on sewage-sick soils that have been maintained for some weeks under favourable conditions of aeration, temperature, etc.*

Number of bacteria, millions per gram of dry soil. Moisture reduced to 16 per cent.

	No lime applied				Lime applied (0·2 per cent.)			
	At start, Aug. 11, 1910	Aug. 24	Oct. 19	Nov. 21	At start, Aug. 11, 1910	Aug. 24	Oct. 19	Nov. 21
ated soil	22	12	35	91	14·4	29	30	38
eated to 98° C.	0·04	18	37	37	0·8	52	53	55
t soil	very few	—	29	42	very few	50	54·5	49
reated with carbon disulphide	2·8	11	136	253	1·5	348	355	168*
reated with toluene	4·2	24	60	441	1·8	181	199	229

Nitrogen present as ammonia, parts per million of dry soil

	No lime added				Lime added			
	At start, Aug. 12	Aug. 24	Oct. 19	Nov. 21	At start, Aug. 12	Aug. 24	Oct. 19	Nov. 21
ated soil	70	50	16	8	70	42	13	16
eated to 98° C.	80	161	345	376	80	198	325	342
soil		193	204	220		159	271	217
reated with CS_2	75	167	228	205	75	157	229	134*
reated with toluene	75	154	103	94	75	171	98	57

Nitrogen present as nitrate, etc., parts per million of dry soil

ated soil	327	346	417	440	327	389	460	435
eated to 98° C.	332	332	335	350	338	346	336	206
soil		43	24	52	50	51	37	74
reated with CS_2	294	306	305	318	316	306	319	317*
reated with toluene	281	289	447	426	309	319	450	500

Sum of ammonia + nitrate, etc., formed, parts per million of dry soil

	No lime added				Lime added			
	Already present at start, Aug. 12	Additional amount formed after			Already present at start, Aug. 12	Additional amount formed after		
		13 days Aug. 24	69 days Oct. 19	102 days Nov. 21		13 days Aug. 24	69 days Oct. 19	102 days Nov. 21
ated soil	397	0	37	55	397	34	66	53
eated to 98° C.	412	81	268	314	418	126	243	130
soil	236	0	0	35	210	0	100	80
eated with carbon disulphide	370	103	163	153	391	72	158	60*
eated with toluene	356	87	194	165	384	106	164	174

* This sample had become very dry during the last four weeks of the experiment.

extent and maintain their high numbers without any sign of falling off; there is also a great increase in the rate of ammonia production. The heated soil as usual behaves differently and throughout the whole of the time only contains a low number of bacteria, although the amount of decomposition is higher than in any other case.

Lime has, at first, a strong depressing action on the bacteria, reducing the numbers in the untreated soil from 22 to 14 millions and in the chemically treated soils from 2·8 and 4·2 to 1·5 and 1·8 respectively. In a very short time, however, the numbers rise, particularly in the soils treated with carbon disulphide and toluene, and the amount of nitrate also increases; neither of these effects is permanent. The effect on the bacteria in the heated soil is quite distinct, but it is so small as to rule out any hypothesis that the unsuitability of the heated soil for bacterial growth is due to the formation of acid substances by heat. The action on the untreated soil is very small and clearly demonstrates that lime does not put out of action the harmful factor, although it does somewhat hasten the decomposition.

There is a distinct qualitative difference between the action of carbon disulphide and that of toluene. Carbon disulphide is the more effective antiseptic and not only reduces the bacterial numbers to a greater extent but even kills certain forms—notably the brown ones—that are partially spared by the toluene. It seems likely on other grounds that the difference in behaviour is due to physical causes; the vapour of carbon disulphide has great power of penetrating the soil and of getting at the organisms; while toluene, with its lower power of penetration, leaves some of the organisms untouched.

TABLE IV. *Percentage of nitrogen present in soils 70 days after partial sterilisation (Oct. 19 samples of Table II used).*

	No lime added	Lime added
Untreated soil	·561	·570
Soil heated to 98° C.	·548	·535
Burnt soil	·524	·524
Soil treated with CS_2	·539	·561
Soil treated with toluene...	·541	·541

In consequence two interesting effects are produced in the toluened soil (1) high bacterial numbers (441 millions) are subsequently reached, the soil being really a partially sterilised soil + a trace of untreated soil (see Table V), (2) nitrification sets up after a time, although it is suppressed in all the other partially sterilised soils. This difference between the two antiseptics is not always observed; under other

circumstances, not yet definitely characterised, treatment with toluene kills all the brown forms and leads to no greater bacterial development than treatment with carbon disulphide.

The increased rate of decomposition in the partially sterilised soils causes as usual a loss of nitrogen, and in consequence they contain a smaller percentage of nitrogen after the lapse of some weeks than the untreated soil, as seen in Table IV.

In our experience this relative loss of nitrogen invariably sets in after partial sterilisation.

The comparison of the new flora arising after partial sterilisation with the old is made by introducing a little of the untreated soil (0·5 per cent.) into the partially sterilised soil. In all cases the old flora has the greater power of multiplication and of effecting decomposition, and the results, given in Table V, show beyond doubt that the effect of partial sterilisation is not to improve the bacteria but to give them a better opportunity of working.

TABLE V. *Comparison of the old bacterial flora with the new.*

	Numbers of bacteria, millions per gram			Nitrogen present as ammonia and nitrate, parts per million of dry soil					
	At start, Jan. 5, 1911	Jan. 14	Jan. 27	As ammonia		As nitrate, etc.		Sum of ammonia + nitrate, etc.	
				Jan. 14	Jan. 27	Jan. 14	Jan. 27	Jan. 14	Jan. 27
reated soil	37	31	31	73·8	90·8	12·3	9·1	86	100
treated with CS_2: new flora		130	110	93·2	139	16·3	7·9	109·5	147
treated with CS_2, then reinfected with 0·5 % untreated soil: old flora		110	475	105	263	11·5	9·2	116·5	272

Moisture = 12 per cent.

The unsuitability of the untreated soil for bacterial growth is not due to any substance that can be washed out, for the aqueous extract of the untreated soil has no depressing effect on bacterial activity in the partially sterilised soil. The extract was made by shaking 200 grams of soil with 500 c.c. of water, allowing to settle for an hour and filtering through paper: 30 c.c. were used for each bottle of 500 grams of soil. No substantial difference is produced in the bacterial numbers nor in the rate of decomposition, although there is a change in the course of the reaction due to the introduction of nitrifying organisms (Table VI). On September 30, however, a more complete bacteriological

examination was undertaken and a separate enumeration was made of the forms not killed by toluene, which may perhaps without serious error be regarded as spores. For this purpose 20 grams of soil were allowed to stand in a weighing-bottle with 1 c.c. of toluene for one hour and then left three hours in a vacuum so that the toluene might evaporate; weighings and attenuations were then made and plates poured in the usual way. The results show a surprisingly small number of forms resistant to toluene in the untreated soil, and further examination has shown this to be a usual characteristic of the untreated soil. These forms accumulate to a much greater extent in the partially sterilised soils, where also there occur a far larger number of forms killed by toluene.

A search for plant toxins decomposable on partial sterilisation also led to negative results. Barley seedlings made even better growth in extracts of untreated soil than in extracts of freshly treated soils; the results of a typical experiment were:—

	Untreated soil	Soil heated to 55° C.	Soil heated to 100° C.	Soil treated with toluene
Weight of shoot, grams	·079	·076	·061	·054
,, root ,, ...	·020	·018	·014	·011
Total per plant.....	·099	·094	·075	·065

The poorer growth in the extracts of partially sterilised soils is under investigation.

The harmful factor resides in the untreated soil and can be transmitted to the partially sterilised soil. When 5 per cent. of untreated soil is added to the toluened soil the rise in numbers due to the addition of the untreated organisms is followed by a gradual fall both in spores and in active forms, and there is a similar falling off in the rate of decomposition. The process of transmission is slow and somewhat erratic and did not complete itself in any of our experiments, but it went sufficiently far to cause a reduction of 25 per cent. in the bacterial numbers. The results are given in Table VI.

The very close resemblance between these phenomena and those observed with ordinary arable soils shows that the factor injurious to bacteria is of the same nature in both cases. It is considered by Russell and Hutchinson that the active protozoa of ordinary soils constitute the harmful factor there, and examination was therefore

TABLE VI. *Effect of adding untreated soil, and the aqueous extract of untreated soil, to toluened soil. Moisture* = 17·5 %.

	Bacterial numbers			Details of Oct. 3 counts	
	After 2 days, May 29, 1911	June 13	Oct. 3	not killed by toluene	killed by toluene
Untreated soil	Plates	44·5	43	11	32
Toluened soil	spoiled	222	272	161	111
Toluened soil + aqueous extract of untreated soil	by	178	281	44	237
Toluened soil + 5 % untreated soil	hot	152	209	117	90
Toluened soil + 10 % untreated soil	weather	211	193	101	92

	Nitrogen present as ammonia + nitrate, etc., parts per million of dry soil								
	As ammonia			As nitrate, etc.			As ammonia + nitrate, etc.		
	May 29	June 13	Oct. 3	May 29	June 13	Oct. 3	May 29	June 13	Oct. 3
reated soil	25	25	12	140	191	327	165	216	339
ıened soil	76	210	277	84	85	157	160	295	434
ıened soil + aqueous extract of untreated soil	92	220	133	82	92	281	174	312	414
ıened soil + 5 % untreated soil	80	180	8	84	130	210	164	310	218
ıened soil + 10 % untreated soil	79	142	7	85	147	346	164	289	353

made for protozoa in sewage-sick soil. They were found in considerable numbers. The usual method of inoculating soil into hay infusion and incubating the mixture fails to discriminate between active forms and cysts. We therefore used a centrifugal method to bring out some of the more active forms. About 10 grams of the soil are well ground in a mortar with 50—100 c.c. of water and the muddy liquid is poured into the cups of the centrifuge, the opposite pairs being balanced by adjusting the quantity of liquid added. The centrifuge is then worked at a fairly high speed till the soil lies at the bottom. The opalescent liquid is next decanted and spun a second time at higher speed; the sediment now sinking to the bottom contains living organisms including many flagellated and other protozoa (some of which are attached to soil particles) and motile bacteria. In examining the sediment under the microscope we obtained the best results by mounting it in a drop of the supernatant liquid. No living forms could be obtained from soil treated with toluene.

The disadvantage of the method is that it reveals only those organisms of the same order of size as bacteria and requires the same power of the microscope in order to be seen. It demonstrates clearly, however, that bacteria are not the only active forms in the soil but that other active organisms also occur[1].

A detailed study of Tables III and VI shows that losses of ammonia sometimes take place over a long period. This happened in Table III on the untreated limed soil and on several of the partially sterilised soils between the 69th and the 102nd day, also in Table V on the toluened soil + 5 % untreated soil. Some volatilisation of ammonia certainly took place, but we obtained evidence of an actual assimilation of ammonia on the untreated soil whenever large amounts of ammonia were present. This is a very unusual action in our experience, and we have never been able to find it on other soils[2]. No assimilation of nitrates took place. The following experiment shows that only 75 out of the 112 parts of nitrogen added as ammonium sulphate were recovered in the form of nitrate and ammonia, the other 37 having disappeared, while recovery of nitrogen added as nitrate was complete:—

	Nitrogen present as NH_3, parts per million of dry soil			Nitrogen present as nitrate, parts per million of dry soil			Nitrogen present as NH_3 + nitrate, parts per million of dry soil		
	At start	After 40 days	After 93 days	At start	After 40 days	After 93 days	At start	After 40 days	After 93 days
Untreated soil alone.....	112	136	78	215	338	383	327	474	461
,, ,, + $(NH_4)_2SO_4$	215	276	164	224	286	372	439	562	536
,, ,, + $NaNO_3$ ...	119	181	101	290	391	443	409	572	544

	Amount of added nitrogen recovered as nitrate and ammonia		
	At start	After 40 days	After 93 days
Untreated soil + $(NH_4)_2SO_4$	112	88	75
,, ,, + $NaNO_3$	82	98	83

[1] Our best results were obtained with a centrifuge running at 4000 revolutions per minute but we could get satisfactory results even with a Gerber centrifuge in which the butyrometer tubes were replaced by test tubes of stout glass. One of us has used this latter method as a class exercise to demonstrate the presence of motile bacteria and other organisms in the soil.

[2] Cf. Russell and Hutchinson, *op. cit.* Vol. III. p. 129, and Russell and Petherbridge, this volume, p. 96.

It did not appear that this assimilation went on in the partially sterilised soil.

Practical application of the results.

The problem we started with was to study the causes of sewage sickness, and our experiments show that they are partly physical and partly biological, and include an abnormal development of a factor harmful to bacteria which is similar in every respect to the factor operating in ordinary soils. This biological factor is therefore not a condition peculiar to sewage treated soils but is of much more general interest as an extreme case of an action going on in all soils. The further conclusion can be drawn that the detrimental factor in ordinary soils is favoured by the water and organic matter present in sewage-sick soils. All our results are in complete agreement with the view that the detrimental factor consists in amoebae and other protozoa.

We have further studied the economic problem of applying our results to the treatment of sewage-sick land, particularly with the view of ascertaining whether partial sterilisation of the soil caused increased purification of the effluent, or whether any modifying factors come into play when the experiment is conducted in the open field.

A number of small land filters were made, 24 inches in diameter and 8 inches in depth when the soil had shrunk to its minimum volume. These were filled with partially "rested" soil some of which was heated rather strongly, some treated with 0·07 per cent. of toluene or of carbon disulphide and some left untreated. The filters were set up in the field and received sewage daily at the same time as it was run on to the land. The effluents were sampled periodically and analysed, with results set out in Table VII.

In the early days of the experiment the effluents from the treated soils were not as good as those from the untreated soil, but after about a fortnight they are seen to be distinctly better. The samples collected on October 20 may be taken as typical of the effluent during the autumn, and show satisfactory purification, especially when the shallowness of the filter is considered. The November sample was taken not long after the filters had frozen. It ran through the soil very quickly in consequence of cracks produced by the frost, and hence was not well purified, but still the treated soils give the best effluents. The February samples were taken so long after frosts that all danger of cracks was past, but the purification effected by the untreated soil is

not so good as in the earlier samples, indicating that sewage sickness is setting in. The treated soils, on the other hand, show no signs of falling efficiency; they are fully as effective as in November, although not quite as good as in the autumn.

TABLE VII. *Composition of effluents from small filters which contained partially sterilised soils. Parts per* 100,000.

	Free and saline ammonia				
	Aug. 12 1910	Aug. 26	Oct. 20	Nov. 19	Feb. 18 1911
Crude sewage	9·50	9·00	5·48	15·00	13·50
Effluent from untreated soil	1·61	1·38	1·84	1·50	3·88
Soil treated with 0·07 % carbon disulphide ...	3·56	3·03	1·26	1·03	1·90
Soil treated with 0·07 % toluene	2·92	0·85	0·40	0·30	0·65
Burnt soil	—	4·86	0·41	0·50	0·50

	Albuminoid ammonia				
	Aug. 12	Aug. 26	Oct. 20	Nov. 19	Feb. 18
Crude sewage	1·40	0·72	1·28	1·85	1·25
Effluents from untreated soil	0·59	0·28	0·22	0·31	0·35
Soil treated with 0·07 % carbon disulphide ...	0·92	0·31	0·16	0·27	0·20
Soil treated with 0·07 % toluene	0·62	0·31	0·16	0·22	0·21
Burnt soil	—	0·57	0·17	0·20	0·06

	Oxygen absorbed				
	Aug. 12	Aug. 26	Oct. 20	Nov. 19	Feb. 18
Crude sewage	5·6	2·32	—	10·14	7·84
Effluents from untreated soil	3·3	1·78	1·08	2·44	3·70
Soil treated with carbon disulphide	3·9	1·28	0·8	2·42	2·25
Soil treated with toluene	3·0	1·38	0·78	2·30	1·23
Burnt soil	—	2·75	1·6	1·36	0·75

A set of filters was made up corresponding exactly with those just dealt with, but receiving in addition lime at the rate of two tons per acre. The result was a rather strongly alkaline effluent much less pure than from the unlimed soils whenever, as not infrequently happened, the alkalinity rose to 9 or 10 c.c. of decinormal alkali per

100 c.c. of effluents. As the alkalinity of the effluent seemed to be the determining factor in the purification, the results need not be discussed here.

In all cases nitrates were present but no estimate of their amount was obtained.

A series of small plots was laid out, one being pared and burnt to a depth of three inches, another treated with toluene (commercial "toluole") applied at the rate of 350 lbs. per acre by means of a Vermorel injector, and a third treated with carbon disulphide at the rate of 350 lbs. per acre. The plots were then sown with turnips. From the outset the plot treated with toluene showed the best and most even lot of plants, the tap root only and not the bulbs being affected by finger-and-toe. The plot treated with carbon disulphide came next, followed by the pared and burned plot, and finally the untreated, the plants on the last two being irregular and much attacked by finger-and-toe. The crops, however, were all good, but those on the treated plots were the best, the weights of total produce being, in thousand pounds per acre:—

Plot treatment ...	Untreated	Pared and burned	Treated with Carbon disulphide	Treated with toluene
Total produce	26·4 30·2	31·8	32·6	36·8

Larger plots of one-tenth of an acre were set out on the College sewage farm at Kingston and drains were laid 18 inches deep—the greatest depth practicable, although insufficient for thorough purification—so that the effluents from each could be tapped. The soil is much heavier than at Kegworth and the sewage differs in character in that it contains a relatively large amount of effluent from the dairy and cheese-making department. The land slopes somewhat, but it was possible to get two pairs of comparable plots, one of which was treated on May 24 with commercial "toluole" at the rate of four hundredweights per acre, the injections being about three inches apart and six inches deep. General inspection on a number of occasions showed that the effluent from the toluole treated plot was the better in each case both in colour and in smell, and the same fact is shown by the analyses of the effluents. In Table VIII it is seen that the treatment effects a marked reduction in the amount of albuminoid ammonia present in the effluent and also somewhat reduces, except in one instance, the high amounts of free and saline ammonia.

TABLE VIII. *Partial analyses of the effluents from the College sewage farm, Kingston. Parts per* 100,000.

		Free and saline ammonia			Albuminoid ammonia		
		July 1, 1911	The same effluent kept till Aug. 1	Nov. 11[1], 1911	July 1, 1911	The same effluent kept till Aug. 1	Nov. 11, 1911
Pair *A*	Untreated	6·83	9·48	6·07	2·50	1·39	3·55
	Treated with toluole...	6·06	7·95	7·65	1·53	1·11	1·30
Pair *B*	Untreated	8·71	11·6	7·4	1·95	1·18	2·22
	Treated with toluole...	6·00	7·11	4·5	1·25	·77	1·75
Crude sewage		6·90	8·78		7·52	9·90	

[1] Satisfactory samples could not be drawn during August and September because the land was laid up to be cleaned from weeds and became so dry (the weather being very hot) that cracks formed and allowed some of the sewage to reach the drains direct. By the end of October the cracks had gone; the November sample represents an effluent that had really percolated through the soil.

These filter and plot experiments have not the same quantitative significance as the laboratory experiments because they are much less under control, but they show that no complicating factors come in on the large scale to change very much the character of the results. No essential difference need therefore be anticipated between the results of the laboratory experiments and of the field trials, and it seems probable that the method will find useful application in sewage farming once the mechanical difficulties of partially sterilising soil on the large scale are overcome. Mr Purvis has pointed out to us some of the economic advantages, such as the possibility of reducing the amount of land necessary for a given population, but into these questions we need not now enter. Our present conclusion is that partial sterilisation may be expected to give useful results wherever the rate of decomposition is limited by the number of bacteria. It removes one of the depressing agents and brings about an increase in bacterial numbers and consequently in the rate of decomposition. Of course if decomposition is limited by some factor other than bacterial numbers partial sterilisation will not necessarily effect any improvement.

Conclusions.

1. Two distinct sets of causes can be traced at work in sewage-sick soils: physical causes that lead to retarded percolation, and a factor detrimental to bacteria.

2. The factor detrimental to bacteria is in every respect similar to that shown by Russell and Hutchinson to exist in ordinary soils. It is put out of action by the same antiseptics and by heat; it is not bacterial nor is it any bacterial product; it is not carried by an aqueous extract of the soil. On the other hand it is transmitted to partially sterilised soils by inoculation with untreated soil. It is not put out of action by aeration or by liming.

3. Its effects are, however, much more pronounced in sewage-sick soil than in ordinary soil. While the bacteria in the untreated sick soil only rarely rise to 40 millions per gram they may rise to as many as 400 millions per gram in the partially sterilised soils. The high amounts of moisture and organic matter in the sewage-sick soil appear to be especially favourable to the harmful factor.

4. Sewage sickness is thus regarded, in part, as an abnormal development of the harmful factor always present in ordinary soils.

5. As in the case of ordinary soils, all the properties of the harmful factor indicate that it is biological and consists in organisms larger than bacteria. Examination of the untreated soil showed the presence of numbers of amoebae and other protozoa, some of which could be separated out in an active form by centrifuging. None were present in the partially sterilised soil. All the evidence at present available points to these as constituting the harmful factor.

6. After the harmful factor is killed by partial sterilisation the bacteria multiply rapidly and rise to high numbers, effecting more decomposition of the added sewage so that a purer effluent is obtained. No complicating factors appear to be introduced when the method is tried on the large scale, and there can be little doubt that once the practical difficulties of partially sterilising large quantities of soil are overcome it will find useful application in sewage farm practice wherever the rate of decomposition is limited by the numbers of bacteria.

PROBABLE ERROR IN PIG FEEDING TRIALS.

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In a recent number of this *Journal*[1], T. B. Wood and F. J. M. Stratton have discussed the probable error of field and feeding experiments, but in the latter case their investigations included only cattle and sheep.

The following work was undertaken as a necessary preliminary to a series of pig feeding experiments which are in contemplation. It is published at once in the hope that the results obtained may be of use to others who may be engaged in planning similar feeding trials.

Among the many records of pig feeding trials, access to which we have been able to obtain, only two series are of any use for the calculation of probable error. In all the other records where any considerable number of pigs were under experiment, their individual weights were not recorded.

In a series of experiments described in the tenth annual report (1911) of the West of Scotland Agricultural College, several batches of 8 pigs were fed for 10 to 11 weeks, each on the same diet. The individual weights at the beginning and end of the experimental period are recorded. From these weights, the probable error on one animal, found by the usual least square method, works out for the different batches of 8 pigs at from 4 to 12 per cent. of the live weight increase. The pigs were of both sexes and of various sizes.

Probable errors have also been worked out from the weights of individual pigs recorded in the Report of the Wisconsin Experimental Station for 1895. Two batches of 10 pigs and three batches of 6 pigs were employed and the time of feeding varied from 9 to 15 weeks. No mention is made of sex and the pigs differed considerably in weight. The probable error on one animal works out at from 6 to 12 per cent. of the live weight increase.

As the results available for examination were so few, it was determined to carry out trials for the express purpose of finding the probable error. For this purpose, eighteen pigs were taken and housed in sets of three in six pens. They were fed on an *ad libitum* ration of sharps mixed into a slop with water. Barley meal was introduced in the ninth week and gradually increased in proportion to the sharps. It was

[1] *J. Agric. Sci.* Vol. IV. Part 3.

arranged that the animals should always feed to repletion, and the troughs were taken out of the pens half an hour after each feeding time. During the first six weeks of the experiment the food was given three times a day, but, later, two meals a day were given. Records were kept of the amount of food consumed in each pen so that, if any trio of pigs failed to clear up their food, less was allowed for the following meal. Water was provided for drinking, but was seldom touched. Once a week, a pound of small coal was given to each pen of pigs.

Thirteen of the pigs were put under experiment on July 31st, 1911, but the other five were not obtained till August 7th, so that the experiment, as far as it concerned the whole eighteen animals, began on the latter date. All the pigs were of the large white breed, and the thirteen which started on July 31st were practically uniform in age and weight, while the other five did not differ greatly from these. All the pigs were castrated males and, at the beginning of the experiment, were on an average ten weeks old.

Weighings were made on July 31st, August 7th, August 26th, and afterwards at intervals of four weeks, the last weighing being on Nov. 21st.

Two pigs died during the experiment from causes which had nothing to do with the conditions of the experiment.

The following table shows the weights recorded.

Weights of pigs in lbs.

No. of pig	July 31st	Aug. 7th	Aug. 26th	Sept. 25th	Oct. 23rd	Nov. 21st
1	35	40½	52	74	102	—
2	33	37½	42	60	93	125
3	40	44½	54	78	103	142
4	44	47½	59	79	97	128
5	43	45	58	83	104	139
6	37	41½	51	75	100	133
7	35	37½	42½	59	89	119
8	34	37½	43½	59	83	112
9	40	44½	53	—	—	—
10	41	46	58	82	103	132
11	35	42½	45½	67	85	109
12	36	43½	52½	75	108	140
13	38	44½	54	77	106	129
14	—	31½	41	63	88	120
15	—	26½	33	47	72	104
16	—	28½	37	57	89	124
17	—	29½	35½	53	74	97
18	—	30½	38½	55	77	105

From these figures the following results were obtained by the least square method:

(1) For the whole number of pigs for the complete period the probable error of one animal was 8 per cent. of the average live weight increase.

(2) For the whole number of pigs, the probable error of one animal in the intervals between the weighings was as follows:

(*a*)	18 pigs for	3	weeks	21·6	per cent. of	average	live weight increase.
(*b*)	17 „	4	„	11	„	„	„
(*c*)	17 „	4	„	13·4	„	„	„
(*d*)	16 „	4	„	9·6	„	„	„

The error, therefore, while greater than that for the whole period, tends to diminish as the pigs get older.

(3) For the eleven pigs which survived out of the original thirteen the probable error of one animal is 7·0 per cent. of the average increase.

(4) In the same way as in (2) we get the probable error of one animal as follows:

(*a*)	13 pigs for	4	weeks	15·7	per cent. of	average	live weight increase.
(*b*)	12 „	„		11·2	„	„	„
(*c*)	12 „	„		13·8	„	„	„
(*d*)	11 „	„		8·0	„	„	„

Here, as in (2), the error diminishes as the pigs get older.

It will be seen that the probable errors calculated in (3) and (4) are little less than those in (1) and (2), showing that accuracy is not greatly increased by uniformity in weight among the animals under experiment.

Uniformity of breed, however, appears to be very important, for in a second experiment on nine animals, some of which were large whites, and some middle whites, the rate of increase of the two breeds differed so greatly that the experiment was discontinued.

From the results of these experiments and those obtained from the Scotch and American figures, it appears that the probable error of one animal in a pig feeding experiment is in the region of 10 per cent. of the average live weight increase.

Now, in a comparative feeding experiment, we aim at taking such a number of animals that the differences may be with certainty attributed to the effect of the two diets and not to normal variation.

It is shown, in the paper on probable error, quoted above, that a difference of 3·8 times the probable error ensures that differences due

to normal variation are practically ruled out of the question. The probable error of an average of n animals is obtained by dividing the probable error of an individual by $\sqrt{n}$. We can therefore construct a table showing the relation between the percentage live weight increase and the number of animals required to ensure that the differences are not due to normal variation.

Percentage difference in live weight increase to be expected under the conditions of the experiment	Number of animals required in each lot. (Fractions counted as the next highest whole number)
50	1
40	1
30	2
20	4
10	15
5	54

CONCLUSIONS.

(1) The probable error of one result calculated from a four week period is large but is diminished as the animals get older and more accustomed to the diet.

(2) This error is much reduced by taking a longer period and 12 weeks may be suggested as the shortest period consistent with accuracy.

(3) The probable error of one animal in a mixed lot of approximately the same age and weight is very little more than that in a lot more closely approximating in these respects.

(4) A table has been prepared to show the number of animals which ought to be taken to show up varying differences with precision. For example, fifteen animals must be taken in each lot where the two methods of feeding are expected to show a 10 per cent. difference.

THE ESTIMATION OF POTASSIUM, ESPECIALLY IN FERTILISERS, SOIL EXTRACTS AND PLANT ASHES.

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It is still no uncommon occurrence when the same sample of a potash fertiliser is submitted to different analysts to find the results returned differing by very large amounts. In one case recently, that came within the writer's experience, the results of two of the best known London analysts differed by nearly 3% in the case of a sample of 90% sulphate of potash. The present paper contains an account of an investigation of the sources of error likely to affect the results in the methods now in use. The conclusion is drawn that the perchloric acid method which in Germany has almost superseded the platinum chloride method (Precht, *7th Intl. Congress, App. Chem.*, 1909, Section I, p. 146) is far superior to the older process not only on the ground of cost, but as being freer from error, simpler and less likely to give differences in the hands of different analysts. This method has been applied to the analysis of soil extracts and plant ashes and the conditions are described, by observing which it is possible to obtain very exact results free from the errors attendant on the use of platinum chloride.

Platinum Chloride Method.

Considerable differences exist in practice as to the concentration of the alcohol used for taking up the syrupy mixture of sodium and potassium platinichlorides in order to separate the former, which is the more soluble. Many chemists adopt 80% alcohol as recommended by Fresenius (*Zeit. Anal. Chem.*, 1877, **16**, 63), but others follow Precht (*Zeit. Anal. Chem.*, 1879, 509) in considering that absolute or "high-percentage" ("hochprozentig") alcohol is better. This is the practice of the Stassfurt chemists: Tietjens in his article on "Potash Salts" in Lunge's *Chem. Techn. Untersuchungs-methoden* (English edition, I, 520) considers the fact that potassium platinichloride is less soluble in absolute alcohol (1 in 40,000) than in 80% alcohol (1 in 25,000) to be an advantage, and that the more correct results obtained by many chemists using 80% alcohol are probably due to a "compensation of

errors." In an important paper by Morozewicz published in 1906 (*Bull. Acad. Cracovie*, 1906, 796) this question has been specially investigated. Morozewicz maintains that if absolute alcohol is used, not only are high results usually obtained with mixtures of sodium and potassium chloride, but that three or four times the quantity of platinum chloride is necessary to keep the sodium in solution as platinichloride as in the case when 80 °/₀ alcohol is employed. The following experiments confirm these statements and show the absolute necessity, when the proportion of sodium chloride is considerable, of using 80 °/₀ alcohol if anything like accurate results are to be obtained.

In the majority of the following experiments the potassium platinichloride precipitate was collected on a filter paper, previously dried in a weighing bottle at 100°, as in the Stassfurt method (Lunge, *Technical Methods*, I, 521). A solution of potassium chloride was used containing 1·0 grm. pure KCl per 100 c.c. 10 c.c. = 0·10 grm. KCl.

A. *Potassium Chloride alone.* Taken 0·10 grm. KCl, 2·5 c.c. of a solution of $PtCl_4$ containing 6·8 grms. Pt per 100 c.c. (= 0·17 grm. Pt; theoretically required = 0·13 grm. for K_2PtCl_6).

TABLE I.

Expt. No.	Conditions	K_2O taken	K_2PtCl_6 weighed	K_2O found*	$\frac{K_2O\ found}{K_2O\ taken}$	Remarks
					°/₀	
1	10 c.c. KCl solution = 0·10 gram KCl, 2·5 c.c. Pt solution	0·0632	0·3310	0·0639	101·1	K_2PtCl_6 taken up with 95 °/₀ alcohol; total vol. of alcohol used for washings = 100 c.c.
1ⁱ	Same ppt. K_2PtCl_6 washed† with 50 c.c. AmCl solution (AOAC) then with 50 c.c. 95 °/₀ alcohol	0·0632	0·3345	0·0646	102·2	
1ⁱⁱ	Ppt. from 1ⁱ washed with 50 c.c. 80 °/₀ alcohol	0·0632	0·3272	0·0632	100·0	
1ⁱⁱⁱ	1ⁱⁱ washed with another 50 c.c. 80 °/₀ alcohol	0·0632	0·3265	0·06305	99·8	
2	Duplicate of 1	0·0632	0·3310	0·0639	101·1	
2ⁱ	Duplicate of 1ⁱ	0·0632	0·3375	0·0652	103·1	
2ⁱⁱ	Duplicate of 1ⁱⁱ	0·0632	0·3270	0·06315	99·9	
2ⁱⁱⁱ	Duplicate of 1ⁱⁱⁱ	0·0632	0·3240	0·0626	99·0	

* Using the Stassfurt factor 0·3056 for converting K_2PtCl_6 to 2KCl; this assumes an atomic weight for Pt = 197·2, not the atomic weight 194·8 which is now generally accepted, but is based on the fact that the precipitated platinichloride dried at 100° is not pure K_2PtCl_6, but contains water of crystallisation.

† The ammonium chloride solution saturated with K_2PtCl_6 was prepared according to the official American (AOAC) method, Bulletin No. 107, U.S. Dept. of Agriculture.

It will be seen that by using 95 °/₀ alcohol even with pure potassium chloride slightly high results are obtained; if the precipitate is washed with ammonium chloride and then with 95 °/₀ alcohol, the results are still higher (owing no doubt to precipitation of AmCl by the concentrated alcohol), but on washing with 50 c.c. of 80 °/₀ alcohol, the results become practically the theoretical. Further washing with another 50 c.c. of 80 °/₀ alcohol lowers the results owing to the K_2PtCl_6 beginning to dissolve in the dilute alcohol.

B. *Potassium Chloride and Sodium Chloride.*

Taken 0·10 grm. KCl, 0·10 grm. NaCl, 5 c.c. Platinum solution (= 0·34 grm. Pt. Required for KCl and NaCl = 0·30 grm. Pt).

0·10 grm. KCl taken = **0·0632** K_2O.

TABLE II.

Expt. No.	Conditions	K_2PtCl_6 weighed	K_2O found	K_2O found / K_2O taken
				°/₀
3	Using 105 c.c. 95 °/₀ alcohol in all for taking up and washing	0·4010	0·0774	122·5
3^{i}	Ppt. in 3 washed with 50 c.c. 80 °/₀ alcohol	0·3250	**0·0628**	**99·4**
3^{ii}	Ppt. from 3 washed with further 50 c.c. 80 °/₀ alc.	0·3237	0·0625	98·9
3^{iii}	3^{ii} again washed with 50 c.c. 80 °/₀ alcohol	0·3200	0·0618	97·8
3^{iv}	3^{iii} again washed with 50 c.c. 80 °/₀ alcohol	0·3160	0·0610	96·6
4	Using 107 c.c. 95 °/₀ alc. for taking up and washing	0·3905	0·0754	119·3
4^{i}	Ppt. in 4 washed with 50 c.c. 80 °/₀ alcohol	0·3250	**0·0634**	**100·4**
4^{ii}	4^{i} washed with additional 50 c.c. 80 °/₀ alcohol	0·3265	0·0631	99·8
4^{iii}	4^{ii} washed again with 50 c.c. 80 °/₀ alcohol	0·3230	0·0624	98·7

It is seen that, even with such small quantities as 0·10 grm. KCl and 0·10 grm. NaCl, by using 105 c.c. of 95 °/₀ alcohol in all, for taking up and washing, very high results are obtained; the 95 °/₀ alcohol was naturally added in small quantities at a time, as in the Stassfurt method, 20 c.c. being used to take up the syrupy platinichlorides. On once washing the precipitate on the filter paper with 50 c.c. of 80 °/₀ alcohol practically correct results are obtained (0·0628 and 0·0634 grm. KCl instead of the 0·0632 grm. taken), but subsequent washing steadily causes loss of weight, owing to the solubility of the potassium platinichloride, and the results become lower and lower with successive washings.

That the high results obtained when 95 °/₀ alcohol is used are due to co-precipitation of sodium chloride with the potassium platinichloride is shown by the fact that on washing the first precipitates 3 and 4 with 80 °/₀ alcohol and evaporating the washings so obtained in a glass

dish, white transparent cubes of sodium chloride are observable under the microscope. Morozewicz has attributed this separation of sodium chloride to the decomposition by absolute alcohol of sodium platinichloride in the sense $Na_2PtCl_6 \rightarrow 2NaCl + PtCl_4$; and shows that when absolute alcohol is used, three times as much platinum chloride is necessary to keep all the sodium chloride in solution in the form of sodium platinichloride as in the case when 80 °/₀ alcohol is employed. In the latter case, however, a slight deficiency of platinum chloride below that required to form Na_2PtCl_6 is not prejudicial to the results.

When using the platinum method, especially in estimating small quantities of potassium as in soil extracts, one of the greatest difficulties is to know precisely when the washing is sufficiently complete to remove the last traces of sodium platinichloride; if washing is too prolonged, serious loss of *potassium* platinichloride may occur—a loss, which when the actual amount of potassium platinichloride weighed is small, may cause considerable error. Experiments were made to ascertain the amount of potassium platinichloride actually passing into solution on washing the pure salt on a filter paper with successive 50 c.c. portions of 80 °/₀ alcohol; the following results were obtained:

1st	wash,	loss	= 0·0015
2nd	,,	,,	= 0·0020
3rd	,,	,,	= 0·0025
4th	,,	,,	= 0·0023
5th	,,	,,	= 0·0022
		Average	0·0021

From Precht's determination of the solubility of K_2PtCl_6 in 80 °/₀ alcohol, if the alcohol passed away saturated, the loss for every 50 c.c. of alcohol would be 0·0020 grm., which agrees closely with the figure obtained above.

This loss of 2 mgrms. for every 50 c.c. of washing alcohol is a serious matter when only small quantities of platinichloride have to be weighed. The uncertainty of the amount of washing required, which is a difficulty in the platinum method[1], is entirely avoided in the perchlorate method, where the loss of perchlorate is practically *nil* when the washing is carried out at first with 95 °/₀ alcohol containing 0·2 °/₀ of perchloric acid, the perchloric acid being finally removed by washing with a very small quantity of pure 95 °/₀ alcohol until the washings no longer show any acidity to litmus paper (see p. 57).

[1] An obvious method of avoiding this difficulty would of course be to use in washing 80 °/₀ alcohol which has been previously saturated with pure potassium platinichloride.

In practice, some analysts evaporate the aqueous solution of the mixed platinichlorides nearly to dryness, then moisten with a small quantity of water and take up with 90 to 95% alcohol, subsequently washing with the stronger alcohol; the moistening with water tends of course to bring the concentration of the alcohol in the first washing nearer the 80% value and thus to render the results more correct than by using absolute alcohol throughout.

One of the greatest difficulties encountered in using the platinum method is met with when analysing sulphates, in which case the whole of the $-SO_4$ has to be *very exactly* precipitated with barium chloride, as, for example, in the Stassfurt method. If there be a slight excess of barium chloride, or, on the other hand, of sulphuric acid, the platinum method gives erroneous results. The perchlorate method, on the contrary, gives perfectly accurate results even when a relatively large amount of barium chloride is present, or a considerable proportion of the potash exists as sulphate. In fact, as will be shown, when the perchloric acid is in large excess the potassium in potassium sulphate itself can be estimated exactly without any treatment whatever to convert the sulphate into chloride.

To sum up: the platinum method is liable

(1) To give *high* results, unless 80% alcohol is used.

(2) To give *low* results when 80% alcohol is used, owing to the relatively high solubility of K_2PtCl_6.

(3) To necessitate great care when sulphates are present; high results may easily be obtained unless the precipitation of the sulphate by barium chloride is very exactly carried out.

In view of the many other real advantages possessed by the perchlorate method and the undoubted fact (as will be demonstrated later) that it gives accurate results without any very special precautions having to be taken, it is contended that the platinum method should be abandoned on account of its uncertainty, cost and special difficulties of manipulation.

Perchlorate Method.

The procedure adopted was as follows: when iron, aluminium or other salts are present, as in ordinary acid soil extracts, ashes, etc., the solution of chlorides is evaporated to dryness in a porcelain dish and ignited for about ¼ hour at a dull red heat so as to throw out iron, etc., as oxides, as in Neubauer's simplified method (*Landw. Vers. St.*, 1905, **63**, 141). The ignition should be continued so long that, on dissolving,

a colourless solution, free from iron, is obtained. [When sulphates are present in large amount, after the liquid has evaporated to dryness, 5 to 10 c.c. of saturated barium hydroxide solution is added to precipitate SO_4. The evaporation and ignition are then completed as usual.] The soluble alkali salts are then extracted with boiling water as completely as possible, breaking up the iron oxide residue with a glass rod during the extraction. The aqueous extract is filtered into a glass evaporating dish ($3\frac{1}{4}$" diam.), care being taken to extract all the alkali salts from the residue; this is usually complete with a volume of hot water sufficient to fill the $3\frac{1}{4}$" dish, but if any doubt exist, another 50 c.c. of boiling water can be used. The aqueous extract is now evaporated *nearly* to dryness after adding 2·5 c.c. of perchloric acid solution, sp. gr. 1·125[1] (20 %); the evaporation must be carried to the point of vigorous evolution of heavy white fumes of perchloric acid. A sand bath not too strongly heated is the most suitable means of effecting evaporation[2]. The soluble perchlorates are now taken up by stirring with 20 c.c. of 95—96 % alcohol, and after settling, the clear solution poured off through a 9 cm. filter paper, which has been dried to constant weight at 100° in a stoppered weighing bottle ($1\frac{1}{2}$" diam. × 2"). 10 c.c. of 95 % alcohol is now added containing 0·2 % of perchloric acid[3], and the insoluble potassium perchlorate transferred as completely as possible by means of it to the weighed filter paper. In washing the last traces of precipitate into the filter paper another 20 or 30 c.c. of the alcohol containing 0·2 % perchloric acid is used, and finally the perchloric acid itself is washed out of the filter as completely as possible by using a minimum of 95 % alcohol. For this purpose the washings are tested, until quite free from acidity, with sensitive litmus paper. Care must be taken that the top edge of the filter paper is washed well. The freedom of the filter paper from perchloric acid is shown by its not blackening during the subsequent drying in the oven at 100°. The use of a glass dish for the evaporation of the solution in the early part of the process greatly simplifies the complete removal of the last traces of the perchlorate precipitate to the filter paper, as these last traces are then plainly visible. In washing,

[1] This quantity is sufficient in most cases, but if the quantity of KCl+NaCl exceeds 0·2 grm., 5 c.c. of perchloric acid or more should be used. It is necessary only to take about $1\frac{1}{2}$ times the quantity of $HClO_4$ theoretically necessary to decompose all the salts present.

[2] If the liquid evaporates to dryness no harm is done so long as there is no loss by spirting. It is only necessary to take up with a drop or two of the perchloric acid solution.

[3] Made up by adding 5 c.c. 20 % perchloric acid to 500 c.c. 95 % alcohol.

a total quantity of 120—150 c.c. of 95 °/₀ alcohol can be safely used without causing any perceptible loss of potassium perchlorate, although so much is not usually necessary unless much NaCl is present. After washing, the filter paper and precipitate are dried in a steam oven for about 20 mins. whilst still in the funnel, the filter paper plus precipitate is then transferred to its weighing bottle and the drying completed until the weight is constant. 1 mgrm. $KClO_4 = 0{\cdot}3401$ mgrm. K_2O.

A Gooch crucible or Soxhlet tube can also be employed for collecting the potassium perchlorate, but if this is used care must be taken that the asbestos layer is sufficiently thick to prevent the very finely divided potassium perchlorate from passing through. With a layer $\frac{1}{8}$" thick perfectly accurate results can be obtained. In rapid working, when a large number of analyses have to be made, the Gooch crucible is preferable to a filter paper.

Experiments.

A. *Very small quantities pure Potassium chloride and sulphate mixed—no sodium.*

TABLE III.

Taken 0·0400 grm. KCl + 0·0100 grm. $K_2SO_4 = 0{\cdot}0307$ grm. K_2O.
With 0·050 grm. Fe present as ferric chloride.
Using 2·5 c.c. 20 °/₀ perchloric acid solution.

No.	Conditions	$KClO_4$ weighed	K_2O found	$\frac{K_2O \text{ found}}{K_2O \text{ taken}}$
				°/₀
1	No barium chloride present	0·0920	0·0312	101·6
2	,, ,, ,,	0·0897	0·0305	99·4
3	,, ,, ,,	0·0910	0·0305	100·6
4	,, ,, ,,	0·0900	0·0306	99·7
5	,, ,, ,,	0·0905	0·0307	100·0
		Average.....	0·0307	100·0

These results show that even when part of the potash is present as sulphate and no barium chloride is used to convert the sulphate into chloride, accurate results are obtained.

The following results were obtained by adding an excess of barium chloride to the solution whilst evaporating to remove the iron so as to convert all the potassium sulphate into chloride; the barium sulphate

formed was left with the insoluble ferric oxide on extracting, whilst the excess of barium passed into the potassium chloride solution. The above results are sufficient to show that such treatment is not actually necessary, but those which follow indicate that if such treatment is adopted in presence of a large proportion of sulphates or sulphuric acid, the precipitation need not be carried out very exactly, but a considerable excess of barium chloride can be present in the solution without interfering in the least. Such an excess would be, of course, utterly prejudicial to an analysis carried out by the platinum method.

TABLE IV.

Taken 0·0400 grm. KCl + 0·0100 grm. K_2SO_4 + 0·05 Fe in the form of ferric chloride.

Using 2·5 c.c. 20 % perchloric acid. K_2O taken = **0·0307** grm.

No.	Conditions	$KClO_4$ weighed	K_2O found	$\frac{K_2O\ found}{K_2O\ taken}$	Remarks
				%	
1	2 c.c. of 1·27 % solution* $BaCl_2, 2H_2O$ = 0·0182 K_2SO_4	0·0875	0·0298	97·1	$BaCl_2$ used nearly double the theoretical quantity to convert all the K_2SO_4 into KCl
2	,, ,, ,,	0·0937	0·0318	103·6	
3	3 c.c. $BaCl_2$ solution = 0·0273 grm. K_2SO_4	0·0911	0·0310	100·9	$BaCl_2$ = nearly three times theoretical quantity
4	,, ,, ,,	0·0889	0·0303	98·7	

* 1 c.c. = 5·0 mg. SO_4.

The following results were obtained using 0·10 grm. pure potassium sulphate (in the absence of iron), evaporating down direct with 2·5 c.c. of 20 % perchloric acid and collecting the perchlorate as usual. They show that under the conditions given the conversion of the sulphate into chloride by barium chloride is quite unnecessary.

TABLE V.

0·100 grm. K_2SO_4 = **0·0541** K_2O. 2·5 c.c. perchloric solution.

No.	Conditions	$KClO_4$ weighed	K_2O found	$\frac{K_2O\ found}{K_2O\ taken}$
				%
1	0·10 grm. K_2SO_4, no $BaCl_2$	0·1575	0·0536	99·1
2	,, ,, ,,	0·1575	0·0536	99·1

B. *Potassium chloride + excess of sodium chloride.*

TABLE VI.

Taken 0·10 grm. KCl + 0·20 grm. NaCl = **0·0632** K_2O.

No.	Conditions	$KClO_4$ weighed	K_2O found	$\frac{K_2O \text{ found}}{K_2O \text{ taken}}$	Remarks
				%	
1	5 c.c. 20 % $HClO_4$ solution	0·1870	0·0636	100·6	The solution of mixed chlorides was evaporated down direct with the perchloric acid solution and treated as usual
2	5 c.c. ,, ,, ,,	0·1855	0·0631	99·9	
3	2·5 c.c. ,, ,, ,,	0·1855	0·0631	99·9	
4	2·5 c.c. ,, ,, ,,	0·1860	0·0632	100·0	
	Average.....	0·06325		100·1	

The results are thus quite accurate in presence of twice as much sodium chloride as potassium chloride; 2·5 c.c. of perchloric solution is quite sufficient under the conditions given to transform all the sodium into soluble sodium perchlorate.

C. *Potassium chloride + excess of sodium phosphate.*

Taken 0·10 grm. KCl + 0·20 grm. Na_2HPO_4, $12H_2O$. Used 2·5 c.c. perchloric solution.

0·10 grm. KCl = **0·0632** K_2O.

TABLE VII.

No.	Conditions	$KClO_4$ weighed	K_2O found	$\frac{K_2O \text{ found}}{K_2O \text{ taken}}$
				%
1	2·5 c.c. 20 % perchloric	0·1880	0·0639	101·2
2	,, ,, ,,	0·1840	0·0626	99·0
3	,, ,, ,,	0·1865	0·0634	100·3
	Average.....		0·0633	100·2

In these experiments the solution was directly evaporated with the perchloric acid. It is seen that the presence of a large proportion of sodium phosphate does not in the least interfere with the accuracy of the results.

D. *Potassium chloride + calcium chloride.*

Taken 0·10 grm. KCl + 0·10 grm. $CaCO_3$ dissolved in dilute hydrochloric acid. The mixture was evaporated direct with 2·5 c.c. of 20 % perchloric acid.

0·10 grm. KCl = **0·0632** grm. K_2O.

TABLE VIII.

No.	$KClO_4$ weighed	K_2O found	K_2O found / K_2O taken
			%
1	0·1850	0·0629	99·6
2	0·1875	0·0637	100·8
	Average.....	0·0633	100·2

E. *Potassium chloride + magnesium sulphate.*

It was found that when magnesium sulphate is present in large proportion the results obtained are quite inaccurate; with 0·10 grm. $MgSO_4$, $2H_2O$ present to 0·10 grm. KCl the results are 5 to 10 % high, and when 0·20 grm. magnesium sulphate is present the results are about 40 % high. In these cases the mixed salts were evaporated directly with the perchloric acid; evaporating the original solution to dryness and igniting, as when iron is present, and then using the aqueous extract for treatment with perchloric acid does not greatly mend matters (Experiments 5 and 6). But by adding 1 grm. of barium hydroxide to the original solution, evaporating, igniting, and then treating the filtered aqueous extract with perchloric acid in the usual way, exact results are obtained (Experiments 7 and 8). Experiments 9 and 10 show that when a solution containing ferric chloride, sodium phosphate

TABLE IX.

Using 2·5 c.c. 20 % perchloric acid. 0·10 grm. KCl = **0·0632** K_2O.

No.	Conditions	$KClO_4$ weighed	K_2O found	K_2O found / K_2O taken	Remarks
				%	
1	0·10 grm. KCl + 0·20 grm. $MgSO_4$, $7H_2O$	0·2505	0·0852	134·8	Directly evaporated with the perchloric solution
2	,, ,,	0·2735	0·0930	147·2	
3	0·10 grm. KCl + 0·10 $MgSO_4$, $7H_2O$	0·2045	0·0695	110·0	Directly evaporated with the perchloric solution
4	,, ,,	0·1980	0·0673	106·5	
5	0·10 grm. KCl + 0·20 $MgSO_4$, $7H_2O$	0·2345	0·0797	128·2	Original solution evaporated, ignited, and extract treated with perchloric acid
6	,, ,,	0·2315	0·0787	124·6	
7	0·10 grm. KCl + 0·20 $MgSO_4$ + 1·0 $Ba(OH)_2$	0·1870	0·0635	100·5	Evaporated with 1 grm. barium hydroxide, ignited, extracted, and treated as usual
8	,, ,,	0·1885	0·0641	101·4	
9	0·10 KCl + 0·10 $MgSO_4$, $7H_2O$ + 0·10 Na_2HPO_4, $12H_2O$ + 0·05 Fe as chloride	0·1860	0·0632	100·0	Evaporated to dryness, ignited, and extract treated as usual. No $Ba(OH)_2$ used
10	,, ,,	0·1845	0·0628	99·4	

and magnesium sulphate is evaporated to dryness, ignited and the extract treated with perchloric acid in the usual way, exact results are obtained without any prior treatment with barium hydroxide; but in treating solutions containing much sulphate it is always safest to add a little baryta. Considerable excess of baryta is without prejudice to the results.

That a large excess of magnesium, when present as *chloride*, is not prejudicial to the results, is shown by the following figures. In practice, this fact is of importance in the analysis of such materials as carnallite and kainite; in such cases, it is only necessary to convert the sulphates into chlorides by treatment with a slight excess of barium chloride, it being unnecessary to remove the magnesium, even when present in large quantity.

TABLE X.

0·10 grm. KCl + 10 c.c. of a solution of magnesium chloride prepared from pure magnesium oxide and containing in the 10 c.c. 0·282 grm. $MgCl_2, 6H_2O$ (equivalent to 0·342 grm. $MgSO_4, 7H_2O$).

0·10 grm. KCl = **0·0632** K_2O.

No.	Conditions	$KClO_4$ weighed	K_2O found	$\frac{K_2O \text{ found}}{K_2O \text{ taken}}$	Remarks
1	0·10 grm. KCl + 0·282 $MgCl_2, 6H_2O$	0·1850	0·0629	99·6 %	Using 2·5 c.c. 20% perchloric acid solution
2	,, ,, ,,	0·1855	0·0631	99·8	,, 5·0 c.c. ,, ,,

F. *Soil extracts.*

The following examples show comparisons of results given by the perchlorate method in the estimation of small amounts of potassium, with those obtained in the usual platinum chloride method as carried out at Rothamsted. The results are expressed in actual grms. of K_2O.

TABLE XI.

No.	K_2O by $KClO_4$ method	K_2O by K_2PtCl_6 washed with 80% alcohol	K_2O by K_2PtCl_6, treated with AmCl solution and then with 80% alcohol (A.O.A.C. Bull. 107)
1	0·0250 grm.	0·0245 grm.	0·0225 grm.
2	0·0178	0·0178	0·0169
3	0·0104	0·0110	0·0097
4	0·0078	0·0084	0·0064
5	0·0076	0·0068	0·0047
6	0·0068	0·0084	0·0062
7	0·0231	0·0204	—

Some experiments were made to ascertain whether any loss of potash was to be feared owing to volatilisation during the ignition of the dry residue obtained by evaporating a soil extract; this ignition was carried out at a very dull red heat, over an ordinary ½" Fletcher's Argand gas-burner, turned about two-thirds on. It was also possible that some potash would be retained by the ignited residue, as was actually found to be the case by Neubauer in the case of soil extracts containing a deficiency of calcium salts; to obviate this he recommended, in such cases, that a little calcium chloride be added (0·2 to 0·5 grm., in the form of pure calcium carbonate added to the acid extract). The experiments made to test these points were carried out with alluvial soils rich in clay and organic matter, which had been found to give difficulty in estimating potassium by the platinum method in use at the Rothamsted laboratory. As a type of this kind of soil, the Orgarswick soil, No. 236 (Romney Marshes), of the survey of Kent, Surrey and Sussex (Board of Agriculture, 1911), may be mentioned; it contains clay 20·3, loss on ignition 11·0 %, $CaCO_3$ 0·42 %, K_2O = 0·66 %. It was found that such soils gave practically identical results with the perchloric acid method carried out as already described, with previous ignition, to those obtained in the survey; on adding, too, a known weight of potassium chloride to the soil extract and then proceeding as usual, practically 100 per cent. of the total potash present was accounted for in the analysis, as the following examples show:

TABLE XII.

No.	Conditions	K_2O taken	K_2O found	$\frac{K_2O \text{ taken}}{K_2O \text{ found}}$ %	Remarks
1	Soil 236 + 0·10 grm. KCl	0·0790	0·0782	99·0	5 c.c. 20 % perchloric acid
2	Soil 52 + 0·10 grm. KCl	0·0842	0·0842	100·0	,, ,, ,,

Many analyses, which need not be recorded here, have shown that, in dealing with practically all soil extracts, treatment with baryta (or barium chloride) is unnecessary, as the proportion of sulphates is generally very small. In analysing artificial plant-food solutions containing magnesium sulphate this treatment is, however, essential.

G. *Ash Analysis.*

The perchlorate method lends itself particularly well to the rapid and exact estimation of potassium in plant ashes, and in the ashes

obtained in evaporating crude liquors in the manufacture of organic acids (tartaric, citric, oxalic). The aqueous extract of the ash is filtered and, if necessary, evaporated to dryness with a small proportion of baryta, to remove sulphates; the residue is extracted with boiling water, filtered and the filtrate treated with 2·5 to 5 c.c. of perchloric acid in the usual manner. Very numerous analyses made by this method have convinced the writer of its rapidity and exactness.

Loss of Potassium Perchlorate in washing. Use of Alcohol saturated with Potassium Perchlorate.

All the foregoing analyses were carried out using 95—96 °/₀ alcohol containing 0·2 °/₀ of perchloric acid for the main portion of the washing, the last traces of perchloric acid being removed by means of the least possible quantity of pure 95 °/₀ alcohol (usually about 5 c.c.). The character of the results shows that by working in this way small error is incurred. But when the method was employed in other hands in the routine analyses of the laboratory, it was found that duplicate analyses frequently differed by several milligrams on the weight of the perchlorate. These differences were ultimately found to be due to too large a volume of alcohol being used in the final washing to remove perchloric acid. Experiment showed that the solubility of potassium perchlorate in pure 95 °/₀ alcohol is relatively high, each 50 c.c. dissolving from 0·0065 to 0·0085 grm. of the pure potassium perchlorate when passed through a Soxhlet tube containing the precipitate. The higher values were obtained when the alcohol was passed through slowly so as to give it a better opportunity of becoming saturated with the salt. The average value obtained in 12 experiments, carried out under conditions approximating to those generally used in washing, gave 0·0073 grm. per 50 c.c. of 95 °/₀ alcohol. On the other hand the amount dissolved under similar conditions by 95 °/₀ alcohol containing 0·2 °/₀ of perchloric acid is very much smaller, being about 0·0012 grm. per 50 c.c. of the alcohol (correction having been made for the 5 c.c. of ordinary alcohol used to wash out the perchloric acid).

It is clear, therefore, that in washing the perchlorate precipitate care must be taken to avoid dissolving traces of it whilst washing out the perchloric acid. To obviate this error, the simplest method is to use, instead of the 95 °/₀ alcohol containing 0·2 °/₀ perchloric acid, pure 95 °/₀ alcohol which has been previously saturated with potassium perchlorate at the temperature at which the actual washing

is carried out[1]. In this case care must be taken *to drain off, as completely as possible, the* 20 *c.c. of alcohol containing perchloric acid* (with which the precipitate was first taken up) before adding the alcohol saturated with perchlorate. If this precaution be not observed, traces of potassium perchlorate are thrown out of the saturated solution on mixing with the alcohol containing perchloric acid and the result is very slightly high. There is no necessity to wash finally with pure alcohol, as the error introduced by the trace of perchlorate present in the saturated alcohol is less than 0·0001 grm.

By using 95 °/₀ alcohol saturated with potassium perchlorate, the advantage is obtained that it is possible to wash with comparatively large volumes of alcohol if necessary (for example 100 to 200 c.c.) without any error being introduced owing to the precipitate dissolving; this is often necessary when other perchlorates, such as those of sodium and barium, are present in large amount. Its use, too, removes all uncertainty as to whether the washing has been efficiently carried out, as it is only necessary, after once weighing, to wash again with 50 c.c. of the alcohol saturated with perchlorate, dry and again weigh. The second weighing should not differ from the first by more than 0·0005 grm. Such treatment is desirable in estimating very small weights of potassium, and numerous analyses, which need not here be quoted, have shown that a high degree of accuracy is hereby attained.

SUMMARY.

It is shown that whereas the platinum chloride method of estimation is uncertain and liable to give varying results, the perchlorate method described is at once more simple in manipulation and more uniform and exact in its results. An improvement has been introduced which consists in washing the perchlorate precipitate with 95 °/₀ alcohol saturated with potassium perchlorate by means of which any error due to the solubility of the precipitate is obviated. This is of importance when dealing with small quantities of precipitate. The following other advantages may here be enumerated:

[1] As the solubility of the perchlorate in 95 °/₀ alcohol somewhat rapidly increases with rise of temperature, it is necessary to saturate the alcohol with perchlorate at approximately the temperature of working. This can easily be done by keeping a Winchester quart full of alcohol in contact with the powdered perchlorate and filtering off fresh quantities just before use. Experience has shown that alcohol saturated on a cold day will dissolve 1 to 2 mgrm. per 50 c.c. when the temperature rises from 75° to 80° F.

(1) *Economy.* In view of the very large excess of platinum required when sodium salts are present and the great cost of the metal at the present moment (£13 per oz.) this is a very real advantage. The troublesome working up of large quantities of platinum residues with the attendant risks of poisoning are avoided.

(2) The presence of barium, magnesium and calcium chlorides and sodium phosphate is without prejudice to the method, and these salts need not be removed. Potassium sulphate can be estimated direct, using a sufficient excess of perchloric acid, without conversion into chloride by means of barium chloride; if, in the analysis of commercial sulphate of potash, the sulphate is converted into chloride by the Stassfurt method, the *exact* precipitation of the sulphate is not imperative as in using the platinum method. There can be excess of either potassium sulphate or barium chloride.

(3) All uncertainty such as exists as to what value shall be taken for the atomic weight of platinum (see for example Precht, *Int. Congress, App. Chem.*, 1909, I. p. 145) is avoided. The calculation is made from the simple molecular ratios $\frac{KClO_4}{K_2O}$, $\frac{KClO_4}{KCl}$, etc.

CIDER SICKNESS.

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The present paper deals with the results of an investigation on a serious and common disorder of cider, generally known as cider sickness. The account here given is concerned with its characteristic features, its cause, and, as far as present knowledge permits, methods for its prevention. The disorder is due to the action of a bacterium. It is proposed to give a detailed account of the organism and its chemical activities in a supplementary paper.

Among the disorders to which cider is liable it is the most dreaded by cider makers in this country and causes the greatest loss, the extent of which has been estimated at several thousand pounds per annum. Its distribution is general in all the cider-producing districts, some of which, however, suffer much more severely than others. The heavy, sweet ciders typical of the best cider districts of Somerset are, for example, much more susceptible to attack than the lighter and brisker ciders of the Hereford type.

The disorder makes its appearance every year in ciders of the current season's making. It is generally during the month of May that the first symptoms of sickness may be observed in ciders most susceptible to the malady. From that time until the end of the summer outbreaks in more resistant ciders occur. The prevailing temperature has much to do with the exact time of the outbreak, a warm spring and early summer tending to cause an early appearance of the trouble, while a cool season checks its onset. The prevalence and intensity of the disorder vary considerably in different seasons, for which the character of the season's vintage as well as the summer temperature must be held responsible. There is little doubt that its occurrence is more widespread than is generally supposed. The features of the disorder are familiar to and quickly recognised by the more progressive makers, but in many cellars no distinction is drawn between it and a renewal of ordinary alcoholic fermentation due to yeast action.

Cider sickness is not confined to this country. It is apparently common in France and probably occurs also to some extent in other cider-producing countries. It is impossible to speak with much certainty on this point and about other matters concerned with manifestations of the disorder abroad, since the references are not sufficiently detailed to possess much diagnostic value. It seems probable that France and England are the only places which suffer severely, since the general type of fruit utilised for cider making in other countries is decidedly more acid in character than in the two countries named. As will be shown later, sharp ciders are not so liable to sickness as those of less acidity.

The malady is undoubtedly not one of recent introduction: but little satisfactory information can be gathered from the older writers upon cider on account of their failure to distinguish it from renewed normal alcoholic fermentation. No English authors appear to have given it special attention, nor have any detailed references to it been found in any of the modern German works on cider. The French literature on the subject is also scanty. Jacquemin refers to it in his *La Cidrerie moderne*, which is one of the most complete recent text-books on cider making, giving a very brief description of it under the name "Maladie de la pousse" and classing it with a disorder of the same name occurring in wines. The latter is familiar to wine makers and has been studied by Pasteur, Gautier, Duclaux, Laborde, Mazé and Pactollet, and other observers. A summary of their work is given by Semichon in his *Traité des Maladies des Vins*. From that account it is clear that, while there are many points of resemblance between cider sickness and the "maladies de la pousse et de la tourne" of wines, the disorders are not identical nor are they produced by the same organism.

We have not been successful in finding any fuller accounts of the disorder nor records of any investigations which have been made upon it: but the literature on cider is not easily accessible, and it is possible that some references may have escaped our notice.

The subject has attracted attention for some seasons past at the National Fruit and Cider Institute and a considerable amount of information with regard to it has been obtained from observations on ciders made there under practical conditions. Notes on the disorder were published in the *Annual Reports* of the Institute for 1906 and 1907. No serious work upon it was, however, attempted until the spring of 1911, when the organism which causes the disorder was successfully isolated in pure culture. Professor J. H. Priestley then

arranged for a part of the work to be conducted in his laboratory at Bristol: and our best thanks are due to him for placing it at our disposal and for his readiness to facilitate the work in every way.

The Characters of the Disorder.

The following description of the characteristic features of the disorder is typical of most outbreaks; but considerable variations occur in individual cases. These will be dealt with in turn.

The earliest symptoms noticed in an attacked cider are a characteristic frothing of the liquor and a sudden and violent evolution of gas. Both bottled and draught cider are liable to attack. The first signs of trouble begin to appear generally early in May. By that time the ciders made during the preceding autumn and winter are fit for consumption, the normal alcoholic fermentation having practically ceased and the liquor being in a more or less quiescent and brilliant condition and almost free from deposit. The preliminary frothing which occurs at the onset of active sickness in bottled cider is slight but persistent, and may almost invariably be taken as a definite sign of the development of the disorder, if it appears in a cider from May onwards. If a bottle is opened at this stage, the contents are ejected with considerable force and so much frothing that it is difficult to collect any quantity of the liquid in a tumbler, the bulk being lost in the form of froth. In a few days sufficient pressure of gas is developed to cause the bottles to explode violently: and unless the corks are quickly released, the whole stock of the cider may be lost. Associated with this fermentative activity is a marked change in the aroma and flavour of the liquor, the original fruity character being lost or, more probably, overpowered by the development of a strong, peculiar and characteristic odour and taste. The sweetness is also reduced and in many cases eventually entirely disappears.

The next stage is the appearance of a haziness or slight turbidity in the hitherto clear liquid. Occasionally it is first observed almost simultaneously with the outbreak of fermentation. Generally it does not become strikingly noticeable until several days or, in extreme cases, weeks later. It increases gradually in intensity, until the liquor eventually attains a thick, milky condition. As the development of the turbidity proceeds, the colour of the cider apparently becomes paler. Part of this effect is doubtless due to the suspension of the creamy-brown particles of the substance causing the turbidity in the

liquid: but an actual loss of colour also undoubtedly occurs. As the turbidity increases, a thick deposit of an amorphous light brown substance is gradually thrown down.

After a lapse of time, in some cases extending to the following year, the liquor clears itself naturally, the whole of the material causing the turbidity being deposited as a crust on the sides and base of the receptacle. The characteristic "sick" flavour and aroma at the same time slowly disappear, and a dry cider of normal, though inferior, odour and taste is left. In this final condition it is drinkable, but its original market value is seriously reduced. Sometimes the turbidity is more persistent and the liquor does not become clear or fit to drink after protracted storage, traces of the "sick" flavour and odour being still retained.

Cider in cask is more liable to sicken than that in bottle. It is very noticeable that any disturbance, such as racking, at the critical season of the year tends to cause sickness to develope more quickly. In some instances it may escape entirely if undisturbed during hot weather, whereas if racked, sickness quickly follows.

Cider which has turned sick is generally apt, after the cessation of the violent fermentation, to acetify much more rapidly on exposure to air than sound cider similarly exposed.

While these features are typical of the disorder in its most complete form, several variations occur.

In some cases the whole series of changes already described takes place; but, instead of the fermentation proceeding until the sweetness is entirely dissipated, it stops prematurely, and the resulting cider then remains permanently more or less sweet

Occasionally, especially in some bottled ciders, fermentation proceeds as usual, but the liquor never passes through the stage of turbidity. It remains clear, but a copious deposit is thrown down. Even the latter character may sometimes be lacking, the deposit being little more than that produced by the same cider remaining in a sound condition.

At times the fermentation itself shows considerable variation in intensity, often being very slight and in rare cases absent entirely, the only visible sign of sickness then being the clouding of the liquor, which is, however, accompanied by the "sick" flavour and aroma.

The development of this flavour and aroma is a feature common to all the instances referred to, and at present must be regarded as the sole constant character associated with the disorder. Instances of

sudden fermentations strongly resembling those occurring in sickness, except for the absence of the characteristic odour and taste, have been observed from time to time: but it has not yet been proved that they are in any way connected with sickness.

In accepting provisionally the occurrence of the characteristic flavour and aroma as the only constant features by which sickness may be diagnosed, attention may be called to certain abnormal instances of the occurrence of these characters. In 1906 among the samples of cider apples submitted to the National Fruit and Cider Institute for analysis was one sent from the Totnes district of Devon. While the juice was being expressed from some of the apples a strong odour of sickness was noticed in the laboratory, and on examining the freshly pressed juice it was found to possess the characteristic odour and flavour of sick cider. Each of the remaining apples of the sample was then tasted, and in every case the flavour of sickness was more or less strongly marked. The apples both outwardly and internally appeared perfectly sound and normal, and there was no trace of disease of any kind present beyond a few specks of the Apple Scab fungus, *Venturia inaequalis*, upon some of the apples. In the tissues of the fruit no bacteria or fungi could be found. The fruit from other trees of different varieties in the same orchard was quite normal in flavour. Specimens of fruit from the same tree have been examined in subsequent seasons, but in no case has the sick flavour been again discerned, the taste of the apples being normal in every respect. Until last autumn this was, as far as we know, the only case of the kind recorded. Certainly nothing similar had been met with among the several thousand samples of apples which were examined at the Institute during the years 1904–10. Last autumn, however, the same feature was observed in a number of different samples of apples analysed at the Institute. The apples affected were of several distinct varieties, quite different in character, and the fruit in many instances was grown in widely separate districts. The conclusion suggested by the facts was that the presence of the sick flavour was not due to the nature or variety of the apple nor to the soil or locality where the fruit was grown, but to abnormal chemical changes occurring during the later stages of the ripening. The flavour was generally most marked as the fruit approached the over-ripe condition. Presumably the abnormal character of the summer of 1911 was responsible for the common occurrence of the phenomenon. In no case could the presence of a diseased condition of the fruit, due either to fungi or bacteria, be associated with the development of the flavour. Probably,

therefore, the correct interpretation is that owing to certain abnormal physiological actions during the course of ripening the same chemical changes of certain constituents of the juice took place within the fruit, which occur in cider during the course of sickness as the result of bacterial action.

The Chemistry of Sickness.

Consideration of the variations in the manifestation of sickness which have been observed shows that the leading changes occurring in cider during an attack of the disorder in its complete form are threefold:

a. The development of a characteristic aroma and flavour.
b. The destruction of sugar accompanied by an evolution of gas.
c. The production of a more or less dense turbidity and deposit.

a. The first change is probably due to the formation of small quantities of volatile ethers, aldehydes, and other organic substances possessing distinctive and powerful aromas. The effect is almost certainly composite and not due to the presence of a single substance. The chances of identification are small, since such bodies when produced during the course of fermentation are generally only formed in minute traces and cannot easily be isolated and identified. However, certain odoriferous substances have been successfully recognised. These include acetaldehyde, ethyl alcohol, and acetic acid. Higher alcohols, aldehydes, and fatty acids (including either butyric or valeric acid, and possibly both) are also present. The aroma and flavour characteristic of sick cider are, however, possibly due mainly to substances other than those already identified. The latter are formed in solutions of dextrose fermented with pure cultures of the sickness bacterium: but the odour and taste of such solutions, while perfectly characteristic, are entirely distinct from those of sick cider. The same remarks apply to other saccharine liquids fermented with the organism. Hence the aroma and flavour associated with sick cider are probably due largely to the formation of some unrecognised substance or substances from constituents present in apple juice and absent in the other liquids tested.

Since the chemistry of the fermentations caused by the bacterium is still under investigation and will be dealt with in detail in a subsequent paper, reference to that part of the subject in the following pages will be confined to the main features which have already been established.

b. During the course of fermentation of a cider in a state of active sickness there is a rapid fall in its specific gravity, accompanied by a marked loss of sweetness. Analysis has shown that this is owing to the destruction of the reducing sugars present in the liquor. The degree to which this destruction is carried differs considerably in individual instances, as will be gathered from the remarks already made with regard to the variability of the disorder. In extreme cases the diminution of the content of reducing sugars may be very slight, or practically complete. The consideration of the quantitative aspect may be conveniently deferred until the section dealing with the behaviour of the organism in sterilised media, since sick cider contains a variety of organisms in addition to the sickness bacterium, and their influence upon the chemical changes cannot be separated from that of the latter.

The reducing sugars present in cider of an age liable to be affected by the malady are almost entirely, and possibly exclusively, dextrose and laevulose. Their decomposition during sickness follows very closely along the lines of normal alcoholic fermentation by yeast, the main products being carbon dioxide and ethyl alcohol. (That the yeasts in the cider may play some part is admitted, but reference to the section dealing with the behaviour of the organism in sterilised sugar solutions will show the activity of the latter in this direction.)

The evolution of gas is rapid and considerable. At least 95 per cent. of the total amount is carbon dioxide, the remainder being mainly, if not entirely, hydrogen. Some recent experiments with nutrient sugar solutions have shown a destruction of sugar accompanied by the production of ethyl alcohol approximately equivalent to 50 per cent. of the weight of the decomposed sugar, without, however, the slightest sign of effervescence or the formation of more than a trace of free carbon dioxide. The significance of this departure from the normal behaviour has not at present been discovered.

The amount of ethyl alcohol produced during sickness may be considerable, and depends primarily upon the amount of sugar present in the cider at the moment of the development of the malady. It is not uncommon to find that a cider containing, for example, about 6 per cent. of reducing sugar and 3 per cent. of alcohol at the onset of sickness will within the course of a week or so lose practically the whole of its sugar and will double its content of alcohol. Indications point to the production of appreciable quantities of higher alcohols. Comparatively large amounts of glycerine are formed.

The most striking feature thus far observed distinguishing the decomposition of sugar during sickness from that by normal alcoholic yeast fermentation is the relatively large amount of aldehydes produced. The presence of acetaldehyde has been definitely recognised: and higher aldehydes in some quantity and formaldehyde are also formed. It is probable that these bodies play a prominent part in sickness phenomena in connection with the production of turbidity.

The decomposition of the sugar also gives rise to the formation of small quantities of fixed and volatile organic acids. Oxalic, acetic, and butyric acids have been found. Traces of lactic acid also may be formed, but tests for succinic acid have given negative results. The total acid production arising from the fermentation of the sugar rarely exceeds the equivalent of ·2—·3 per cent. malic acid. The actual changes in the acidity of the cider are somewhat complicated, since the organism apparently acts upon the malic acid present: and in moderately sharp ciders the total acidity may show a decrease during sickness in spite of the formation of some acids from the sugars.

c. The turbidity and deposit produced as the result of cider sickness are due partly to the increase in number of the organisms present in the cider. The increase in the bacterial content of the liquor is particularly noticeable. The main cause, however, is undoubtedly the formation of an insoluble substance or mixture of substances. Microscopical examination of a drop of the liquid reveals the presence of innumerable minute granules or resin-like droplets, frequently aggregated together in groups which both in size and form may easily be mistaken for colonies of bacteria of the coccus type. On heating the cider the turbidity to a large extent disappears owing to the solution of this material, which reappears again in its original form on cooling. Complete solution of the material is also effected by alcohol, if it has not been long precipitated. On long standing in the cider its solubility in alcohol appears to diminish considerably, possibly owing to changes in its constitution.

Its nature has not yet been fully investigated, but evidence already available points to its origin from the tannins, and possibly other related bodies, to which the colour of cider is largely due. For instance, its formation is generally most abundant in deeply coloured ciders containing a relatively large amount of tannin, and partial decolorisation of the liquor usually accompanies its appearance. From analogy with certain disorders of wines, such as *la pousse* and *la casse*, some such relationship might be anticipated. It is evidently produced from

constituents of cider not present in any of the ordinary nutrient solutions used for the cultivation of the organism, since no corresponding substance has been observed in the artificially prepared culture fluids.

Several facts suggest that it is formed as the result of the action of the aldehydes produced during sickness on the tannins or allied bodies in the cider. Pending definite proof, however, detailed discussion of the point may be postponed.

The susceptibility of different types of Cider.

Reference has already been made to the difference in the susceptibility of individual ciders to sickness. The records of all the ciders made at the National Fruit and Cider Institute during the past seven years serve to throw considerable light upon this aspect of the problem. These ciders have been made from selected varieties of vintage apples, which, instead of being mixed together prior to cider making according to the usual practice, have been dealt with separately. It has, therefore, been possible to ascertain the relations between the chemical composition of the variety, including variations due to soil and other factors, the type of cider produced from it, and the liability to sickness.

Cider apples may be divided into three classes according to the composition of their juices, viz.:

a. Sharp varieties, the juices of which normally contain more than ·45 per cent. of malic acid.

b. Sweet varieties, with juices containing normally less than ·45 per cent. of malic acid and less than ·2 per cent. of tannin.

c. Bittersweet varieties, yielding juices which contain normally less than ·45 per cent. of malic acid and more than ·2 per cent. of tannin.

As a general rule it is the ciders made from the two latter classes of apples which are susceptible to sickness, those made from the first class being resistant to the malady and usually escaping it entirely. The acidity of the cider is, therefore, evidently an important factor: and the records of individual examples prove that, *ceteris paribus*, the higher the acidity of the cider the more resistant it is to the disorder. The same rule holds good for ciders made from mixtures of fruit.

Since in some districts apples of low acidity predominate and in others sharp varieties are more abundant, it is evident that this is one reason why some localities suffer more than others. The kind of soil on which the fruit is grown also affects the acidity as well as the rate of fermentation of the juice, the importance of which is indicated in the following paragraph.

The amount of residual sugar in the mature cider is also important, sweet ciders suffering severely from sickness and well-fermented dry or nearly dry ciders rarely being noticeably affected. The quantity of sugar is dependent partly upon the method of treatment of the liquor during fermentation, but primarily upon the natural rate of fermentation of the juice. The degree of sweetness of a cider unsweetened by the addition of sugar may generally be taken as a very fair approximate guide to the natural rate of fermentation of the original juice, rapid-fermenting juices yielding under ordinary conditions dry ciders and slow-fermenting juices treated similarly giving sweet ciders.

Hence the greater susceptibility of sweet ciders may be due either to the presence of sugars in some quantity or to the slow rate of fermentation of the juices. Probably both factors are concerned. The presence of some sugar is necessary for the development of sickness to an appreciable extent; but the actual amount is in itself probably immaterial, although the results of the malady are most pronounced when the quantity is large. Since, however, the alcoholic content of the liquor is, roughly speaking, inversely proportional to that of sugar, and alcohol acts unfavourably on the growth of the organism, it follows that the amount of sugar present does in practice have some bearing upon the susceptibility to attack, independently of its relation to the rate of fermentation. The latter in turn, apart altogether from its bearing on sweetness, has much to do with predisposition to the disorder. In general, leaving out of account for the moment considerations of acidity influence, slow-fermenting juices yield susceptible ciders and rapid-fermenting types resistant ones. The explanation which suggests itself is that the yeasts are less well nourished in the former instances than in the latter owing to partial nitrogen starvation, and that consequently they are unable to dominate so thoroughly the flora of the cider, with the result that other organisms, including the sickness bacteria, have a better chance of development. (It may here be stated that the sickness organisms have in many cases been proved to be present in the freshly pressed juice. Probably this original infection rather than contamination at later stages is responsible for most outbreaks.) A vigorous primary fermentation by the yeasts should therefore be encouraged, although it is not invariably successful in warding off sickness, if the cider retains much unfermented sugar.

The liability of slow-fermenting and sweet ciders to sickness constitutes the most serious feature of the malady to practical cider makers, since they are generally of better quality than rapid-fermenting types and have a decidedly higher market value.

The storage temperature of the cider has also an influence upon the development of the malady. The warm season of the year is the signal for its appearance, and the hottest summers generally cause the greatest trouble. If a cider liable to sickness which has been stored at a cellar temperature of about 12° C. is placed at about 25° C., sickness usually appears in a few days, while a control sample left at the original temperature may escape entirely.

Tannin has been generally regarded as possibly the most important constituent of cider for the prevention of the development of its various disorders. In the case of sickness, however, there is clear evidence that its antiseptic properties are ineffective. The malady attacks liquors containing relatively high percentages of tannin as readily as those containing small amounts, if other conditions are equal.

Table A contains statistics of a few representative ciders of various types, illustrating the remarks made above as to the influence of the character of the cider upon its susceptibility to sickness. The specific gravity figures showing the rate of fermentation were taken from samples of the juices kept at a temperature of 27° C. The figures in large type indicate the appearance of sickness, the first in each instance denoting the point at which the disorder was first observed. The sudden drop in gravity between that and the previous record, succeeding a period of marked slackening in the rate of the primary fermentation, points clearly to the onset of the disorder independently of any other accompanying signs.

TABLE A.

Variety	Composition of fresh juice.			Course of fermentation, showing specific gravity at end of						
	Specific gravity	Malic acid %	Tannin %	2nd day	4th day	6th day	8th day	10th day	12th day	14th day
Ashton Foxwhelp	1·053	1·28	·376	1·048	1·031	1·021	1·013	1·008	—	—
Ashton Long Stem	1·052	1·05	·286	1·045	1·036	1·032	1·028	1·027	1·027	1·027
Yellow Styre	1·059	·72	·184	1·043	1·024	1·015	1·007	—	—	—
Kingston Black, A	1·065	·65	·204	1·057	1·050	1·046	1·043	1·041	**1·032**	—
,, ,, B	1·051	·60	·168	1·036	1·028	1·023	1·021	1·020	1·020	1·020
Lady's Finger	1·046	·58	·096	1·031	1·015	1·010	—	—	—	—
Horner	1·059	·36	·228	1·048	1·036	1·030	**1·026**	**1·016**	—	—
Morgan Sweet	1·046	·23	·160	1·041	1·007	1·001	—	—	—	—
Slack-ma-girdle	1·049	·19	·116	1·043	1·040	1·037	**1·027**	**1·021**	**1·014**	**1·008**
Royal Jersey	1·058	·20	·440	1·052	1·043	1·038	1·033	1·032	**1·022**	**1·010**
Twistbody Jersey	1·058	·22	·352	1·049	1·043	1·038	1·033	1·028	1·022	**1·012**
Improved Broadleaf	1·045	·26	·446	1·042	1·034	1·017	—	—	—	—
Upright French	1·052	·32	·460	1·036	1·014	—	—	—	—	—

Sickness in Perry.

Perry, as might be anticipated from its close relationship as a beverage to cider, is also liable to attacks of the disorder. The above account of sickness as it affects ciders applies in almost every particular to perry also, even such characteristic features as the aroma and flavour produced by the malady being identical, or practically so. There is no occasion, therefore, to give a separate detailed account of it in relation to perry.

The Organism of Sickness.

Prior to this investigation it had been generally assumed that sickness was caused by the action of a specific organism or a group of organisms. In the early stages of this work definite proof was soon forthcoming, since sterilised ciders of a suitable type quickly showed all the symptoms of the disorder after infection with a few drops of actively sick cider; while, if the cider used for infection was sterilised before addition, no signs of sickness developed. A similar negative result occurred, if antiseptics were used in the place of sterilisation.

Biological examination of a sick cider has shown that its flora is very varied. The most abundant organisms are bacteria of several types. Many living yeast cells of diverse form are also present. The appearance of the latter, however, is not of a character to suggest that they are the cause of the malady; nor are they as numerous as would be expected if they alone were responsible for the trouble.

The attempt to isolate from sick cider an organism capable of reproducing the disorder was for a long time unsuccessful. None of the yeasts isolated possessed that property: and the only bacteria of note observed were acetic forms. Many series of fractional plate cultures were made from various sick ciders, both beer-wort, apple juice, and cider gelatine and agar media being used. Eventually last spring the sickness bacillus was successfully isolated from a set of beer-wort gelatine plates extra thinly sown from cider which had just begun to show the first symptoms of the disorder. The plate cultures in this case were kept at 22° C.; and it was not until the ninth day after infection that the colonies of the organism first became visible under a simple lens as minute dot-like growths. By that time the colonies of the other organisms present had developed so strongly that, if it had not been for the exceptionally thin sowing, those of the required bacterium would have been overgrown by the others and would have

escaped observation. On the 11th day isolation was possible. From the outset it looked probable that the organism was the one sought, since the number of its colonies far exceeded those of yeasts and acetic bacteria. Infections were taken from four of the colonies, two proving impure and the other two yielding pure cultures of a short rod-like bacterium, which showed itself in due course to be the same form in both cases. Further series of plate cultures were made from the two latter, the same type of colony developing in every instance, their purity thus being attested. From the latter series of cultures the pure stock cultures used in subsequent experiments were taken. Periodic repurification has been effected in the same way, a check thus being kept upon the validity of the results.

The earliest stock cultures were streaks on beer-wort gelatine. From them tubes of sterile beer-wort and cider were infected. After two or three days at 27° C. the former showed signs of active fermentation, but the latter remained apparently unaffected. The beer-wort fermentation did not at first, except for the production of a very frothy head, suggest sickness, the typical flavour and aroma being absent. The addition of a few drops of this actively fermenting wort to sterile cider was, however, sufficient to set up a vigorous fermentation in the latter within 48 hours at 27° C., a very frothy head being formed in this case also and a strong aroma and flavour of cider sickness developed. In course of time a thick milky turbidity was also formed. There was, therefore, no doubt then that the organism causing the malady had been obtained and that it had the power of producing all the phenomena of sickness in sterilised cider without the assistance of other organisms.

It is not proposed to describe here in detail the characters of the bacterium. Reference will be mainly restricted to those having a more or less direct bearing upon cider sickness.

In young cultures on most media, liquid and solid, the organism grows in the form of short, actively motile, rod-like cells, occurring singly or joined in pairs. The rods are generally about two-thirds as long as broad, the length ranging about 2 μ and the breadth about 1 μ. Their ends are slightly rounded. In old cultures the cells are frequently more elongated, and involution forms are common. The latter are very striking on some media, their length extending frequently to 200 μ and their breadth being considerably reduced. The two ends in such cases swell up to globular structures 25 μ or more in diameter. Forms of a dumb-bell shape are also common. No spore formation has yet been observed.

The organism is facultatively anaerobic. It grows on a variety of solid media, including most nutrient gelatines and agars, potato, carrot, and parsnip: but in no case is the amount of growth anything more than extremely limited, even at the optimum temperature and under either aerobic or anaerobic conditions. The rate of growth is also extremely slow. This limited and excessively slow growth on solid media is a very marked feature of the organism.

Growth is more abundant, both in solid and liquid media, in the presence of carbohydrates than in their absence.

The character of the growth on solid media is a creamy white, somewhat slimy mass, if the medium is on the moist side: if dry, the streak is non-spreading, rather darker in tint, and slightly cartilaginous in texture.

In liquid media containing carbohydrates growth is comparatively active, and is accompanied by more or less vigorous fermentation if dextrose or laevulose is present. The fermentation of laevulose appears to be much less active than that of dextrose. Saccharose, maltose, and lactose solutions never show signs of active fermentation, but occasionally the evolution of a few gas bubbles has been observed.

The gas given off during the fermentation of dextrose consists almost entirely of carbon dioxide. A small amount of hydrogen is also evolved, but quantities larger than 5 per cent. of the total gas production have not been noted. Ethyl alcohol is formed in some quantity, nearly 5 per cent. being produced at times from a 10 per cent. dextrose solution. A limited amount of acid is also formed. Acetic, oxalic, and butyric acid occur in small amounts. In a solution of commercial glucose alone (specific gravity 1·030) after eight days at 25° C. the specific gravity was reduced to 1·005, the alcohol formed amounted to 3·88 per cent., and the acidity was increased by the equivalent of ·07 per cent. malic acid.

A marked aroma, resembling somewhat that of decaying lemons in the earlier stage of fermentation and changing to a decidedly acid character in the later stages, accompanies the fermentation of dextrose: but on no occasion when sugar-containing liquids other than cider or perry have been used has any aroma resembling that typical of cider sickness been developed.

A temporary turbidity of the fermenting dextrose solutions occurs during the active stages of fermentation, but this quickly subsides at the cessation of fermentation. It is due apparently entirely to the cells of the organism in suspension and has no relation with that developed during cider sickness. In hopped beer-wort, on the other

hand, turbidity quickly appears and is very persistent. The presence of tannin in this case suggests that the production of the characteristic turbidity of sickness may be related to the occurrence of that substance.

The acidity of the medium has an important influence upon the extent of growth of the organism. It grows best in neutral or very slightly acid media, and its development is entirely inhibited in the presence of much acid. The most complete details with regard to the influence of acidity have been obtained from ciders in which the degree of acidity was varied by partial or complete neutralisation with caustic soda in some instances and calcium carbonate in others. The ciders used for this purpose were of the extremely acid type, the quantities of malic acid present ranging between 1 and 1·5 per cent. Where the acidity was reduced by neutralisation to points below the equivalent of ·3 per cent. malic acid, growth of the organism was rapid and vigorous: between ·3 per cent. and ·5 per cent. it was moderate: above ·5 per cent. conditions were obviously unfavourable and growth was irregular: above 1 per cent. it ceased. These results obtained by direct infection of sterilised cider correspond regularly with observations on ciders under normal conditions where the disorder has been allowed to develop naturally. There is some evidence to show that the organism may be capable of acclimatisation to the presence of considerable quantities of acid, and sickness has been produced in sterilised ciders containing as much as ·9 per cent. malic acid. The resistance of the organism to the effect of acid and other unfavourable conditions depends very materially upon the vigour of the cells at the time of infection of the medium and upon factors influencing their nutrition.

Table B illustrates the effect of the organism on ciders in one of the series in which the acidity was varied by neutralisation with calcium carbonate. It will be noted that fermentation set in most rapidly where the acidity was lowest, and that the most acid sample was practically unaffected. The end-point in each of the other cases was approximately the same, fermentation ceasing suddenly for some unknown reason while the gravity was still relatively high. The total acidity, expressed in all cases in terms of malic acid, shows interesting variations, increasing in the ciders where it was low at the start and decreasing somewhat in the examples at the other end of the table. The results clearly indicate that the bacterium decomposes some of the malic acid originally present, the destruction being marked in the lower members of the series by a larger formation of other acids.

The organism grows best at comparatively high temperatures. Its

temperature limits for growth correspond closely with those ascertained for the development of sickness under practical conditions. The optimum temperature lies about 30° C. Between 20° C. and 35° C. growth is active. Above the latter point the rate slackens considerably, and no development has been observed above 40° C. Below 20° C. growth is also slower, and between 12° C. and 15° C. it is extremely slow. Below 12° C. there is hardly any appreciable development. The organism is killed by exposure to a temperature of 55°—60° C. for five minutes.

TABLE B.

Cider. Ashton Long Stem (specific gravity 1·036)	Specific gravity after infection			Acidity (expressed as malic acid °/₀) after infection		
	2nd day	5th day	8th day	2nd day	5th day	8th day
A. Acidity exactly neutralised with $CaCO_3$	1·019	1·012	1·012	·07	·13	·13
B. Acidity reduced to ·19 °/₀ malic acid by partial neutralisation with $CaCO_3$	1·021	1·012	1·012	·27	·27	·28
C. Do. ·45 °/₀ malic acid	1·021	1·012	1·012	·45	·44	·43
D. Do. ·69 °/₀ malic acid	1·024	1·014	1·011	·61	·55	·53
E. Natural acidity, ·94 °/₀ malic acid	1·036	1·036	1·036	·92	·92	·92

Infection Experiments with Sterilised Ciders.

Many experiments with sterilised ciders of different types in which sickness has been artificially induced by infection with pure cultures of the bacterium have been made, and in all cases the sickness has been in every respect similar to that which occurs naturally in the same ciders. The results of trials with ciders and perries of different types to determine their susceptibility to sickness and to ascertain the conditions which favour its development corroborate those which have already been stated as the outcome of observations on the disorder as it occurs under natural conditions. There is, therefore, no necessity to discuss in detail individual experiments nor to repeat the conclusions drawn therefrom.

The results with artificial infection do not, however, always coincide absolutely with those obtained under natural conditions. In some cases artificially infected ciders of comparatively high acidity have turned

sick, when the malady does not develope in the same ciders under natural conditions. This diversity in behaviour is accounted for partly by the fact that in artificial infections cells of the organism in most vigorous condition are used for inoculation, and partly because the heaviness of the infection largely determines the result. If such ciders are infected very lightly, sickness does not invariably follow, whereas a heavy infection causes sickness. Another possible factor is that under natural conditions the bacteria have to develope in a medium containing active organisms of other kinds, and they may be affected directly or indirectly by their presence. Where artificial infections have been made, on the other hand, the ciders used have previously been sterilised and thus the effect of other organisms is excluded.

Methods for prevention of Sickness.

This investigation of the disorder has suggested several lines of treatment for its prevention.

The occurrence of the bacterium is evidently widespread, and there is good evidence for believing that it is introduced into the cider direct from the fruit when the latter is milled and pressed. Whether it is a soil organism which reaches the fruit at the time of gathering, or whether like the cider yeasts it is normally found on the surface of the ripened fruit, is not at present known. It may be assumed with considerable probability that it is present in most ciders from the start, and that its subsequent history depends very largely upon the nature of the cider itself.

Measures for the prevention of the disorder, therefore, fall into two groups, those concerned with the elimination of the organism from the juice and those devoted to the production of a cider unfavourable to their development.

Under the former head the washing of the fruit prior to milling first merits attention. Washing with cold water has been repeatedly tried. While the results are better on the whole than those from unwashed fruit, they are nevertheless irregular and cannot be relied upon absolutely. Experiments on washing with hot water are now being tried, the results from which are not yet available. The work of Warcollier on the washing of the fruit with an antiseptic wash, such as very dilute formaldehyde, proves that juice practically free from any living organisms can be obtained: and possibly the method might be of service in this connection. While, however, it might be effective in

the hands of a careful worker with a knowledge of chemistry, it may be doubted if it would be judicious to recommend such procedure to cider makers in general.

Pasteurisation of the freshly pressed juice or mature cider also deserves consideration. Unfortunately under most conditions this form of treatment impairs the flavour of the cider.

To restrict infection as far as possible the sterilisation of all vessels and appliances which have been in contact with sick cider is to be recommended.

It must, however, be recognised that at present really satisfactory means for the elimination of the organisms from the juice or cider are lacking, and that the most efficient means of protection consist in the production of a liquor unfavourable for their development.

Since the presence of sugar is necessary for their growth, complete fermentation of the cider to dryness is effective: but it is not feasible in many cases on account of the extensive demand for sweet cider. It has, however, been found that, if juices of the slow-fermenting type are blended with others which ferment at a rapid rate, the product ferments moderately quickly and greater resistance to the disorder is secured than if the former are left unblended.

Another method favouring resistance is to raise the acidity of the cider to about ·5—·7 per cent. of malic acid by suitable blending. It is not practicable to increase the acidity of the beverage much beyond those limits, since the flavour becomes unpleasantly sharp.

It has also been found that the addition of a vigorous culture of yeast in the later stages of the fermentation of a susceptible cider has a beneficial effect, probably owing to the sickness bacteria suffering by competition with the active yeast rather than to the reduction of sugar during the increased fermentation, since there may still be a surplus of the latter after fermentation has been checked.

Fermentation and storage of the cider at as low a temperature as possible are obviously desirable.

The addition of an antiseptic such as salicylic acid is effective, but cannot be recommended on other grounds. Ciders which have been sulphured, are said to be strongly resistant, even after oxidation of practically the whole of the free sulphur dioxide.

In the case of bottled ciders it has been found that susceptible liquors if bottled very early in the season, *e.g.* in late January or February, frequently escape, whereas the same ciders bottled at the usual time in April almost invariably succumb. Perhaps the cause of

the difference in behaviour rests with the yeasts, which are much more active at the earlier period and charge the cider comparatively quickly with carbon dioxide then.

Although not invariably certain in their results, the measures recommended have been found in practical tests to give a very considerable degree of success: and there is fair promise that further experiments on similar lines under practical conditions may result in the elaboration of a sure method for holding the disorder more or less completely in check.

INVESTIGATIONS ON "SICKNESS" IN SOIL.

II. "Sickness" in Glasshouse Soils.

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Previous investigations in this laboratory have shown that partial sterilisation of soil leads to increased productiveness. In attempting to apply this method on the large scale two courses were open. The more obvious was to seek for methods cheap enough for use in the field, and then to conduct a number of field trials to determine which was the best; this was almost certain to prove a tedious and expensive business and would not necessarily lead to a successful issue. The alternative plan, and the one we adopted, was to find classes of growers who could afford to use our present methods of partial sterilisation and who would be willing to do so. However restricted their number of crops might be we knew that the cost of the process must fall once it was applied in commercial growing, so that the range over which it was applicable would soon begin to widen; a further advantage was that from the outset we should be gaining experience of the working of partial sterilisation in practice. Fortunately we met with a large tomato and cucumber grower in the Waltham Cross district who put us in touch with the class of growers we wanted: in this way we came across the problem of sickness in glasshouse soils which forms the subject of the present communication.

It has long been known among growers that soils suffer a change under the intense system of culture prevalent in glasshouses, and gradually become unfitted for the continued growth of a crop. This deterioration—technically known as "sickness"—is perhaps best seen in commercial cucumber houses, where it may be so marked as to necessitate the soil being thrown away after a single season's use. It

also occurs in tomato houses, but it sets in more slowly, so that the soil may last for four or five seasons. Other instances are known, but we have confined ourselves to these two cases, which are typical of the rest, because we have at hand in the Lea Valley a large area under glass where cucumber and tomato sick soils occur in abundance.

The difference in the effective life of the soil in the two cases is probably not connected with any feature of the plant but with the conditions of growth. A commercial cucumber house in the Lea Valley is run at a very high pitch. The "borders" in which the plants grow are made up of alluvial pasture soil mixed with an equal weight[1] of straw manure and a liberal addition of bone meal. After the high temperature of the fermentation has subsided the soil is maintained by artificial heat at about 80° F. (26·7° C.) and is kept so moist that water can be squeezed out simply by the pressure of the hand. Each week during vigorous growth more manure is added, commonly cow manure water and artificials, while at frequent intervals dressings of lime or chalk and of new soil are given. Enormous crops are obtained, but at the end of the season—September or October—the soil ceases to be effective and even after lying uncropped till February, when the next season's borders are made up, it cannot be used again but has to be thrown out, its enormous manurial residues being sacrificed. The amount of the annual loss cannot be estimated, but it must be very great; at one nursery alone we saw over 5000 tons of this soil, which on analysis proved to be richer than farmyard manure (Table I). The new soil wanted for each season's work has to be purchased and carted, often from a considerable distance.

Tomato houses are run at a much lower pitch. Dung is put into the bottom spit during trenching, but little or no organic manure is added to the top spit in the first instance, fertilisers being used only when the fruit has set and has attained the size of peas. Care is taken to avoid excess of manure and water throughout, the temperature also is kept down, 65° F. being usually aimed at. It is commonly agreed that soil will not profitably carry tomatoes for more than three seasons, but growers who grow in the ground and not in pots cannot afford to throw out the old soil. Instead they trench the ground every second or third year, carefully burying the top spit and bringing up the bottom spit; of late years it has become customary to apply an antiseptic such as a dilute emulsion of carbolic acid or of some tar oil to the top soil before burying it; sometimes also antiseptic is applied to the bottom spit after it has been brought up. Under these conditions the soil

[1] Some growers use less, but this is a common mixture.

seems to last indefinitely and "sickness" never sets in; we know of one house where tomatoes have already been grown continuously and successfully for eleven years without any sign of deterioration; here the trenching has been done annually and the antiseptic treatment has been very thorough.

"Sickness" is commonly accompanied by insect and fungus pests, perhaps the most frequent being *Heterodera radicicola* which produces the swellings on the roots both of cucumbers and of tomatoes—the so-called "club."

A comparison of the conditions of cucumber and tomato houses with other cases that have come under our notice leads to the conclusion that "sickness" of soil is associated with high organic matter content, high water content, and relatively high temperature. Where all these conditions are simultaneously present, as in cucumber houses, "sickness" quickly sets in; where only two are present, as on soils heavily dosed with sewage, it takes a longer time (see p. 27); it is even slower to appear in the poorer, drier and cooler tomato soils, where it may reveal itself only by the disease organisms, and finally, going still further down the scale of intense culture, it is no longer heard of in arable soils except in the case of leguminous crops which may form a wholly different case. We can, in fact, trace a perfect gradation from the extreme case of the cucumber house to the ordinary arable field soil.

It has been shown by Russell and Hutchinson that ordinary field soils contain a biological factor detrimental to bacteria, the effect of which is to keep down the bacterial numbers and the rate of production of plant food. This result suggests a simple explanation of "sickness"; the conditions being favourable to high bacterial development also favour a corresponding but slower (p. 98) development of the destructive organisms, till finally, when after many disturbances the new equilibrium sets in, the bacterial efficiency of the soil has fallen too low to maintain the high rate of production called for in a commercial glasshouse. On this hypothesis "sickness" is simply an instance of the limitation of the bacterial population that goes on in every soil, and it should be amenable to treatment by partial sterilisation.

Experiments showed that this expectation was well founded. After partial sterilisation "sick" soils behaved in precisely the same manner as normal arable soils, but to a much more marked extent. The fauna of the soil was simplified, amoebae, ciliates and higher forms being killed, the bacterial numbers rose considerably, the rate of production of plant food increased and larger crops were obtained.

But this simple hypothesis is incomplete because it takes no account of the parasitic or disease organisms present in sick soils. The conditions of the glasshouse industry favour a transfer of organisms from one glasshouse to another, while the conditions of a cucumber house are often not unlike those of a moist chamber in an incubator, so that once an organism appears it may multiply very rapidly. Thus the soil in the cucumber borders becomes inhabited by a remarkably varied population: growths of myxomycetes and other low vegetable forms can be seen on the surface, while active amoebae, eelworms and other low animal forms can be got out by simple centrifuging. No particular disease organism occurs invariably so far as we know, but there is generally evidence that plants in sick soils have been attacked by some or other organisms: whether this is one of the primary causes of the sickness or whether it is only a secondary effect consequent on a weakened physiological condition of the plant we are not in a position to say. In a tomato house the living population is less complex, but it includes many disease organisms.

These disease organisms are an undoubted factor in soil "sickness," indeed we might have considered them the sole agents, as Kühn did long ago in beet sickness on the Continent[1], and Bolley has recently done in the flax and corn-sick soils of Dakota[2], had we restricted ourselves to vegetation experiments. But while the destruction of the disease organisms by partial sterilisation might be supposed to account for the increased growth of crop it does not account for the increased bacterial population and rate of production of plant food, unless we suppose that the bacteria in the "sick" soils have been suffering from some disease. There is nothing absurd in this idea, but it is difficult to reconcile with the fact that addition of bacteria from the "sick" soils to the partially sterilised soils leads to further increases, and not decreases, in bacterial population and in the rate of plant food production (p. 97). Only when "sick" soil itself is added to the partially sterilised soil (our destructive organisms not being contained in the water extract) does any reduction set in.

In the scattered and fragmentary literature dealing with "sickness" various other hypotheses have been put forward, two of which are of sufficient importance to merit attention. It has been supposed

[1] *Bied. Centr.* 1880, 859–865.

[2] H. L. Bolley, "Conservation of the purity of soils in cereal cropping," *Science*, 1910, **32**, 529–541; also Bulletins of the Agricultural Experiment Station for North Dakota, Nos. 50, 55 and 87.

(1) that the "sick" soils gradually acquire some substance toxic to plants,

(2) that they acquire a substance toxic to bacteria.

We have gone carefully into the question of soluble plant toxins and failed to find any evidence of their presence in these soils. Cucumbers grown in an aqueous extract of a "cucumber-sick" soil showed no signs whatever of being poisoned, although other experiments proved that they are very sensitive to traces of poison in the solution. They started at least as well as plants grown in extracts of partially sterilised soils from which the harmful factor has been put out of action, and only fell behind when the food supply was nearing exhaustion. Further, they kept a healthier colour than plants grown in a culture solution, and they did better than plants grown in water alone, although in the latter case root growth started more quickly (Figs. 3 and 4, Plates III, IV, V).

If plant growth were limited by a plant toxin we should expect to find unabsorbed nitrates and ammonia in the soil after the plant has finished growth. This, however, we failed to do: at the end of the experiment the untreated soil is depleted almost as completely as any of the others. We must thus attribute the sickness in the first instance to the diminished bacterial activity we find in the sick soils.

Two consequences follow from this diminished bacterial activity. The decomposition processes go on more slowly, so that relatively low quantities of the simple nutritive decomposition products are present whilst relatively high amounts of complex decomposition products occur. There is evidence that some of these latter may be directly harmful to the plant, although they are not sufficiently soluble to appear in the aqueous extracts. As soon as the bacterial activity increases the soil conditions become more suitable to the plant.

Our experiments thus lead to the conclusion that at least two factors are concerned in soil "sickness": a falling off in bacterial activity and an accumulation of plant parasites and disease organisms. The factor keeping down bacterial numbers possesses identical properties with that present in arable soils; we must provisionally regard it as being the same. No bacterio-toxin could be found; the experiments showed that the factor is biological and non-bacterial. All these harmful organisms are more readily killed than bacterial spores, and partial sterilisation has the effect of leaving a clear field for the bacteria and a healthy soil for the plant. The treatment is effective for some time, since it is known (p. 98) that the re-establishment of the organisms destructive to bacteria is a slow matter, and the plant makes such vigorous growth on

partially sterilised soils that it is less liable than before to take disease even if the organisms get in.

Partial sterilisation therefore appears to be the proper method of dealing with "sick" soils. It is not entirely new. Certain large growers have for some years been in the habit of steaming their old cucumber soil or treating it with carbolic acid to kill "club" and other disease organisms, while many tomato growers treat their soils annually with antiseptics for the same reason. But apart from these special purposes the method is not in use, and we have extended our experiments to ascertain the lines on which large scale trials should be made with the end to make the treatment a regular part of commercial greenhouse practice.

Of the methods of partial sterilisation investigated, exposure to a temperature of 96°–98° C. for two hours has proved the most effective because it not only kills destructive and parasitic organisms, including *Heterodera*, but also effects a certain amount of decomposition, thus lightening the subsequent work of the bacteria and bringing about certain secondary results, notably a great development of fibrous root. This treatment is practicable on the large scale[1]. A temperature of 55° C. maintained for 3 hours suffices to kill destructive and parasitic organisms and thus to remedy sickness, but it does not effect the secondary changes. Toluene and carbon disulphide, the antiseptics we have most studied, produce satisfactory improvement in the bacterial numbers and the rate of formation of plant food, but they do not kill all the *Heterodera* nor do they bring about the useful secondary changes. But for practical purposes an application of antiseptic would prove more convenient than heat if the purely mechanical difficulty of distribution were overcome. The antiseptic should be some cheap substance or mixture of substances that can (1) put out of action the factor injurious to bacteria, (2) kill *Heterodera* and the spores of parasitic disease fungi in the soil, (3) when its work is done disappear from the soil by volatilisation, oxidation or other decomposition, leaving no permanent bad effects behind, (4) if possible lead to the same fine root development as a temperature of 98° C. We find at least six substances or classes of substances that more or less satisfy the first three conditions:

(1) formaldehyde;

(2) the lighter hydrocarbons of the tar oils: benzene, toluene, and the higher homologues present in the so-called light solvent naphtha and heavy solvent naphtha;

[1] See Russell and Petherbridge, *Journ. Board of Agric.* 1912, XVIII. 809–826.

(3) the heavier hydrocarbons of the naphthalene fraction;
(4) the tar acids: phenol, cresylic acid, etc.;
(5) the tar bases: pyridene and the homologues;
(6) calcium sulphides.

Pl. II, fig. 1 and Pl. III, fig. 2 show some of the results obtained. Of these the lighter hydrocarbons are not convenient for large scale work because of the cost of transport (they being classed as "dangerous goods" by railway companies) and the difficulty of application. The others are more suitable; they are carried at ordinary rates and are readily put on to the soil because they are or can be made miscible with water. Further investigation is necessary to discriminate between the various substances. None of them, however, causes the fine fibrous root development obtained on heated soil; for practical purposes steaming therefore remains the best of all the methods we have tried.

Experimental.

The "sick" soils were collected in lots of two cwts. or more from large nurseries in the Lea Valley, chiefly in the region between Enfield Lock and St Margarets, and examined by the three methods that have given us the most useful results in other fertility investigations, viz. analysis, determination of the rate of production of ammonia, and vegetation experiments. The analytical determinations call for no special comment, their object being to ascertain whether the soil presents any marked peculiarity or deviates to any notable extent from the normal.

The rate of production of ammonia is measured by the sum of the ammonia and nitrate present at a given time. For this purpose a quantity of the soil is mixed very uniformly and divided into a number of 800 gram lots, some of which are left untreated as controls, while others are treated in the various desired ways. The different lots are then moistened so that all shall contain the same amount of water, they are put into litre bottles stoppered with cotton wool plugs, and kept in a dark cupboard. At definite intervals bottles are taken out for the determination of nitrates and ammonia. In untreated soils the amount of ammonia is only small because its rate of formation under normal conditions is slower than its rate of conversion into nitrates[1]. The determination affords a measure of the decomposability of the nitrogenous organic matter of the soil and an index of the amount of nitrogen

[1] This *Journal*, 1910, **3**, 233.

likely to become available for a crop grown under similar circumstances. The method is based on the assumption that the ammonia and nitrates are neither being assimilated by micro organisms nor suffering any other decomposition except conversion one into the other. Control experiments have always to be made to find out whether the assumption holds good. An independent series of experiments is therefore started in which the soil is mixed with known weights of ammonium salts and of nitrates and kept under the same conditions as in the main series, determinations being made from time to time to see whether the added salts are recovered entirely as ammonia and nitrate.

Recovery appears to be complete in ordinary arable soils and in glasshouse soils, but as the method is open to criticism pot experiments are always made as a control. Equal amounts of the variously treated soils are put up into pots, non-leguminous crops are grown, weighed and analysed. The weight of nitrogen in the crop is obviously the final measure of the amount of nitrogen the plant could extract from the soil unless growth was limited by some other factor, in which case some of the nitrates will be left behind unassimilated. After the crop is removed, determinations are therefore made of the residual nitrates in the soil. In normal cases they amount only to about five parts per million of soil.

The analysis of the soils.

Fairly complete examinations have been made of two cucumber sick soils and three tomato sick soils, and less complete examinations of three other soils; the analytical results are shown in Table I. The cucumber sick soils are extraordinarily rich, especially in phosphates and potash, as might have been expected from their manuring. A considerable amount of calcium carbonate is also present and there is no sign of acidity; indeed, so far as our experience goes, the soil in the cucumber house even when described as sour is alkaline because of the ammonia that is evolved from the manure. "Sickness" therefore cannot be attributed to lack of lime or plant food or to the presence of acids in the soil.

The tomato soils are poorer, especially *MT* and *SB*, which have been cropped for five and seven years respectively; the richest of the series *RC* 1 had been a cucumber soil, but was steam sterilised and mixed with new turf and one-eighth its weight of straw manure and used for tomatoes. In these cases also there is no sign of acidity or deficiency of lime.

TABLE I. *Percentage composition of sick soils, air dried.*

	Cucumber sick soils			Tomato sick soils				
	*A**	*B**	*OxL*	*RC* 1	*SC*	*MT*	*M*	*SB*
Moisture	7·3	5·1	3·1	2·3	1·2	1·8	·9	1·7
Loss on ignition	18·7	19·9	16·9	8·7	7·9	6·0	8·0	6·0
Total nitrogen	·75	·72	·63	·37	·33	·26	·32	·22
Nitrogen as nitrates	·027	·021	·017	·016	—	·005	—	—
,, ammonia	·0015	·0015	·0015	·0005	—	·0004	—	—
Calcium carbonate	1·07	·92	1·93	·57	—	·97	—	·63
P_2O_5 sol. in conc. HCl	·56	·73	·55	·39	·31	·39	—	—
P_2O_5 sol. in 1 % citric acid	·36	·47	·29	·21	·17	·23	—	—
K_2O sol. in conc. HCl	·54	·50	·26	·45	·46	·44	—	—
K_2O sol. in 1 % citric acid	·12	·10	·18	·08	·06	·07	—	—

* *A* and *B* were from nurseries not far apart, the soil being made up from the same pasture land ("turf"). In spite of the admixture of an equal weight of dung and the frequent dressings of manure the organic matter and nitrogen have not increased very much. The composition of the original "turf" was:—

Moisture	Loss on ignition	Nitrogen	$CaCO_3$
6·26	13·49	·59	·15

P_2O_5 (in HCl)	P_2O_5 (in citric acid)	K_2O (in HCl)	K_2O (in citric acid)
·16	·016	·39	·08

The nitrogen forms about 4 per cent. of the organic matter instead of the 3 per cent. found in ordinary arable soils; in the pasture soil from which *A* and *B* were made up the value is 4·4.

The effect of partial sterilisation on the rate of production of plant food.

(*a*) *Cucumber sick soils.* Treatment of the soil with 0·5 per cent. of toluene causes an instantaneous production of ammonia, the amount of which rises from 15 to 25 parts per million of soil (Table II). Heat, especially to a temperature above 90° C., has a much more drastic effect, increasing the ammonia to 74 parts per million and causing so much decomposition of organic matter that the water extract of the soil, instead of being a golden yellow colour, becomes brown. There is, however, no evidence that the solubility of the phosphorus or potassium compounds in citric acid solution is increased by the treatment.

When the soil is moistened and stored ammonia is gradually formed and is converted into nitrates in the untreated soil, and the soil heated to 55°, but accumulates unchanged in the soil heated to 98°, till finally more than 300 parts per million are present, an extraordinary high

TABLE II. *Effect of heat and toluene on cucumber sick soils.*

A. Soil *OxL*. Immediate effect.

	Untreated soil	Partially sterilised soil		
		Treated with toluene	Heated 2 hrs. at 98°	Heated 2 hrs. at 55°
N as ammonia, parts per million of dry soil	15	25	74	38
N as nitrate, parts per million of dry soil	274	272	267	277
P_2O_5 sol. in 1 % citric acid, per cent.	·29	·30	·28	—
K_2O " " "	·18	·17	·17	—

Subsequent effect, 45 % moisture being present.

	N present as ammonia, per million of dry soil			N present as nitrate, per million of dry soil			Total, ammonia + nitrate, per million of dry soil		
	At start	After 15 days	After 43 days	At start	After 15 days	After 43 days	At start	After 15 days	After 43 days
Untreated soil...	15	16	16	274	279	315	289	295	331
Soil heated to 98°	74	264	338	267	239	288	341	503	626
" " 55°	38	11	13	277	333	392	315	344	405

B. Soil *OxL*. 42·4 % moisture present.

	N present as ammonia, per million of dry soil		N present as nitrate, per million of dry soil		Total, ammonia + nitrate, per million of dry soil	
	At start	After 43 days	At start	After 43 days	At start	After 43 days
Untreated soil	13	21	315	347	328	368
Soil treated with toluene	23	134	295	325	318	459
" " CS_2	20	143	282	326	302	469
Soil heated to 98° for 2 hrs.	64	255	323	342	387	597

amount that we have seen equalled only in partially sterilised sewage sick soils.

Another experiment is recorded in the second part (B) of the Table. A slow decomposition has gone on in the untreated soil, the total nitrate and ammonia increasing by 40 parts per million,

equal to 12 per cent. of the amount initially present. A much more rapid decomposition occurs in the soils treated with toluene and carbon disulphide, and a marked accumulation of ammonia takes place; the total nitrate and ammonia now increase by 140 and 170 parts per million, these gains being equal to 44 and 55 per cent. of the respective initial quantities.

The soil heated to 98° shows a still greater rate of decomposition and ammonia accumulation, the total amount of nitrate and ammonia now rising to 600 parts per million, a gain equal to 54 per cent. of that originally present in the heated soil and 82 per cent. of that originally present in the untreated soil.

A simultaneous set of experiments showed that the accumulation of nitrate and ammonia in the partially sterilised soils is not the result of throwing out of action some agency present in the untreated soil that removes nitrates and ammonia. Some untreated soil was divided into three portions, two of which received small quantities of ammonium sulphate and sodium nitrate respectively; after 43 days the nitrate and ammonia were estimated; the results are given in Table III.

TABLE III. *Amounts of ammonia and nitrate recovered from soils receiving known weights of* $(NH_4)_2SO_4$ *and* $NaNO_3$. *Parts per million of dry soil.*

	N present as ammonia		N present as nitrate		N present as ammonia + nitrate		Percentage of added N recovered
	At start	After 43 days	At start	After 43 days	At start	After 43 days	
Soil *OxL.*							
Untreated soil alone.....	13	21	315	347	328	368	—
,, ,, $+(NH_4)_2SO_4$	118	21	325	519	443	540	150
,, ,, $+NaNO_3$ ...	14	21	453	485	467	506	99
Soil *RC.*							
Untreated soil alone.....	5	3	108	136	113	139	—
,, ,, $+(NH_4)_2SO_4$	88	3	122	245	210	248	109
,, ,, $+NaNO_3$ ...	5	4	215	240	220	244	105

In all cases recovery is complete and we may conclude that the increased quantities of ammonia and nitrate in the partially sterilised soils are the results of greater bacterial activity there.

Although bacterial counts were not made in our experiments they were taken in parallel experiments made in conjunction with Dr Hutchinson, and they showed a marked increase in numbers in the toluened soil, but no increase in the untreated soil—indeed there was usually a fall. The following are typical results:

	Millions of bacteria per gram of soil			
	At start	After 13 days	After 25 days	After 70 days
Untreated soil	65	41	22	23
Soil treated with toluene...	8	187	128	182

It is thus evident that the untreated soil contains a factor limiting the numbers and activity of the bacteria but put out of action by toluene, carbon disulphide or heat. This factor is not a soluble toxin, for it is not present in the water extract of the untreated soil, in fact addition of such a water extract increases the numbers of the bacteria[1]. Nor is the factor any group of bacteria, for on reintroducing the original flora into the partially sterilised soil[2] there is not a fall, but a rise in numbers. It is not a toxin produced by bacteria, since it shows no signs of setting up in partially sterilised soils kept free from infection, in spite of the high degree of bacterial activity. The data on which these conclusions are based have been ascertained in conjunction with Dr Hutchinson and are dealt with fully in another paper, for convenience typical results are given here showing the conditions in the soil "*OxL*" 15 days after treatment:

	Bacteria, millions per gram	N as ammonia	N as nitrate	N as ammonia + nitrate
Untreated soil	82	17	343	360
Soil treated with toluene	245	99	281	380
Soil treated with toluene + water extract of untreated soil.....	408	101	303	404
Soil treated with toluene + 0·5 % untreated soil	287	107	291	398

Finally the factor can be introduced by adding somewhat larger quantities of untreated soil, *e.g.* 5 per cent., when the bacterial numbers

[1] The water extract contains bacteria.

[2] Done by infecting with 0·5 % of untreated soil.

go down, indeed the smaller inoculation (0·5 per cent.) sometimes suffices, but so far as our experiments have gone it is not introduced in any other way:

	Bacterial numbers, millions per gram		Ammonia + nitrate, per million of dry soil	
	After 15 days	After 70 days	After 15 days	After 70 days
Toluened soil + 0·5 % untreated soil ...	233	255	162	389
,, ,, + 5 % ,, ,, ...	243	117	176	270

The factor is slow growing and takes some time to assert itself.

Thus a complete parallelism can be traced between these sick soils and the normal arable soils investigated by Russell and Hutchinson, and the conclusion seems irresistible that similar agents are at work in both cases keeping down the bacterial numbers, but their effect is more marked in these rich sick soils than in the poorer arable soils.

The increased bacterial activity of the partially sterilised soils is not wholly expended in the production of plant food. There is a considerable loss of nitrogen from the partially sterilised soils relative to the untreated soils: the percentages after three months were:

In the untreated soil	·853
In the soil treated with toluene	·837
,, ,, CS_2	·818
,, heated to 98°	·800

Other experiments showed that the denitrifying organisms still survive in the toluened soils and effect an immediate reduction in the amount of nitrate directly the air supply is cut off by overwatering. It is therefore wrong to assume, as is sometimes done, that the beneficial effects of partial sterilisation arise from any suppression of bacterial decompositions causing loss of plant food; as a matter of fact all bacterial decompositions appear to be accelerated.

The search for soluble toxins.

We have made a careful search in sick soils for soluble substances toxic to plants, but the results have been wholly negative. A water extract of the sick soil was made by stirring up the soil with ten parts of water, allowing to stand for about 16 hours, and then decanting off

the turbid liquid into the culture vessels. In the first series of experiments the liquid was filtered, but later on this operation was omitted lest the filter should cause any complication. Cucumbers were then grown in these extracts.

Pl. III, fig. 3 *a* shows the effect on the plants observed after 5 days, when ordinary distilled water was used. In 1 (food solution) growth has started, but not very well, in 2 (sick soil extract) the plant is doing remarkably well, in 3 and 4 (extracts of partially sterilised sick soils) growth is only beginning. Ordinary distilled water, however, is somewhat toxic to plants. Another experiment was therefore made in which the whole of the water used for the food solution and the soil extract was distilled in a silver still and collected in glass, so as to get as pure a liquid as possible. At first the plants in the untreated soil extract were at least as good as those in the other extracts, but after a fortnight those in the toluened soil extract began to grow rather more than the others, finally the toluened soil and heated soil extracts gave somewhat larger crops than the untreated extract. The figures are given in Table IV and show that the maximum difference was only 14 per cent. The extracts have not all the same initial composition since both toluene and heat cause a certain liberation of ammonia from the soil: thus some of the extracts contained in parts per million:—

	Cucumber sick soil "*OcL*"			Tomato sick soil "*SB*"		
Extract made from	Free and saline ammonia	Albuminoid ammonia	Nitrate	Free and saline ammonia	Albuminoid ammonia	Nitrate
Untreated soil ...	0·92	3·2	17·8	0·45	1·84	10·6
Toluened soil.....	1·82	2·8	15·6	0·65	1·80	11·7
Soil heated to 55°	3·38	3·6	—	0·13	1·49	10·6
,, ,, 98°	3·54	4·5	—	0·98	1·95	11·1

These figures are reflected in the growth of the plant: while in the early stages the untreated soil extract proved at least as favourable as the others, it sometimes gave rather poorer results later on, when the supply of food was beginning to be exhausted.

A set of cultures was made in which tap water was used[1]. This proved a very good medium for plant growth, and plants started in

[1] Harpenden tap water is hard, containing a good deal of calcium bicarbonate in solution.

it rather more quickly than in the soil extracts. Again, however, the untreated extract was at least as good as the partially sterilised extracts. (Pl. IV, fig. 3 *b*.)

Finally, more concentrated extracts were tried, one part of soil being taken to two of tap water. The results were of the same kind as before, the plants in the untreated soil extract starting fully as well as in the others, and falling off only when the food supply began to be an important factor.

That the cucumber plant is susceptible to traces of poison in culture solution is shown in another experiment with *OxL* soil (Pl. IV, fig. 3 *c* and Table IV), where the food solution and extracts were made with ordinary distilled water which in our laboratory is prepared in a copper

TABLE IV. *Growth of cucumbers in aqueous extracts of untreated and of partially sterilised "cucumber sick" soils.*

		Soil extracts			
			Partially sterilised soils		
	Food solution	Sick soil	Treated with toluene	Heated to 55°	Heated to 100°
i. Soil *A*. Silver distilled water used.					
Wt. of dry matter produced—Root	·0375	·0395	·0742	—	·0646
Shoot	·287	·1868	·1845	—	·1878
Total, grms.	·3245	·2263	·2587	—	·2524
Length of root, cms.	21·6	49·4	63·1	—	59·9
ii. Soil *OxL*. Tap water used (Pl. IV, fig. 3 *b*).					
	Water alone				
Wt. of dry matter produced—Root	0·25	0·4	1·3	1·8	—	1·3
Shoot	0·9	1·9	4·7	4·0	—	4·2
Total, grms.	1·15	2·3	6·0	5·8	—	5·5
Wt. of green shoot	6·1	25·9	44·3	34·7	—	37·6
iii. Soil *OxL*. Ordinary distilled water used (Pl. IV, fig. 3 *c*).						
Wt. of dry matter produced—Root	·0085	·027	·025	·042	·022
Shoot	·110	·219	·163	·231	·183
Total, grms.	·1185	·246	·188	·273	·205
Length of root, cms.	3·1	18·2	23·5	28·1	15·9

still, and therefore contains minute traces of copper. Little or no growth occurred in the food solution although growth was normal in the soil extracts. The retardation sometimes noticed in the extracts of heated or toluened soils can in like manner be attributed to some toxin, possibly excess of ammonia, produced by the treatment. Our interest, however, is in the untreated soil extract; in no case could we find that this extract was less suitable to the early stages of growth of cucumbers than the extract of partially sterilised soils. We have therefore no reason to suppose the presence in the "sick" soils of a soluble plant toxin that is absent from the partially sterilised soils.

The following considerations militate against the view that the effect of partial sterilisation is to remove a toxin from the untreated soil:

(1) Cucumber seeds are very sensitive to unfavourable conditions but they germinate fully as well in sick soil as in partially sterilised soil.

(2) Young seedlings are also very sensitive but they make perfectly satisfactory growth in sick soil till the food supply becomes an important food factor.

(3) Washing a sick soil with much water does not improve it as a medium for the germination of seeds and the growth of seedlings.

The partial sterilisation of tomato sick soils.

The results of the experiments with tomato sick soils are of precisely the same kind as were yielded by cucumber sick soils. Table V shows that the immediate effect of partial sterilisation is to increase the amount of ammonia in the soil, but not to increase the solubility in 1 per cent. citric acid of the potash or the phosphoric acid. Subsequently there is a marked increase in the rate of production of plant food and in the bacterial numbers.

As in the case of the cucumber soils, addition of traces of untreated soil to partially sterilised soils led to still further increases in bacterial numbers and in the rate of decomposition. Thus the original bacterial flora is more potent both for multiplication and decomposition than the new flora developing after partial sterilisation: when the new and the old are placed under equally favourable conditions by growing both on partially sterilised soils, the old proves to be the more effective. We therefore cannot attribute the beneficial effects of partial sterilisation to any change in the type of the bacterial flora or to any supposed

TABLE V. *Effect of heat and toluene on tomato sick soils.*

Soil *MT*. Immediate effect.

	Untreated soil	Partially sterilised soils		
		Treated with toluene	Heated 2 hrs. at 98°	Heated 2 hrs. at 55°
N as ammonia, parts per million of dry soil	4	5	19	10
P_2O_5 sol. in 1 % citric acid, per cent.	·20	·20	·20	—
K_2O " " "	·075	·070	·071	—

Subsequent effect, 14 % of moisture being present.

	Ammonia, parts per million of soil			Nitrate, parts per million of soil			Ammonia + nitrate, parts per million of soil		
	At start	After 32 days	After 114 days	At start	After 32 days	After 114 days	At start	After 32 days	After 114 days
Untreated soil	4	8	8·5	46	54	57	50	62	65·5
Soil treated with toluene	5	44	35	44	56	58	49	100	93
Soil heated to 98° for 2 hours	19	53	78	59	67	74	78	119	152
Soil heated to 50° for 2 hours	10	5	6	51	62	72	61	67	78

Soil *RC*. 23 % of moisture present.

	Ammonia		Nitrate		Ammonia + nitrate		Bacterial numbers, millions per gram	
	At start	After 46 days	At start	After 46 days	At start	After 46 days	At start	After 46 days
Untreated soil	6	4	83	104	89	108	48·5	66
Soil treated with toluene	6	24	81	99	87	123	4·4	120
" " CS_2	6	44	82	83	88	127	1·7	110

stimulus. It is necessary to insist on this point because some bacteriologists still assume that such a change or a stimulus takes place, although they offer no evidence in support of the assumption.

These bacteriological studies were made in conjunction with Dr Hutchinson and the details will be found in another paper written jointly with him. The following typical results may be given here,

showing the numbers of bacteria in millions per gram of the variously treated soils:—

	At start	After 21 days
Untreated soil	9·3	36
Toluened soil	3·6	73
,, ,, +0·5% untreated soil	—	123
,, ,, +aqueous extract containing bacteria from untreated soil	—	131

The results with the aqueous extract show that no soluble bacterio-toxin occurs in notable quantity in the untreated soil.

Attempts to find a soluble plant toxin were also fruitless. The extracts were made as before from the sick soils and also from partially sterilised soils. Tomato seedlings were used in the earlier experiments, but they were given up because they proved unsuitable for water cultures; while they lived, however, they fared quite as well in the sick soil extracts as in the others. In later experiments barley seedlings

TABLE VI. *Growth of barley in aqueous extracts of untreated and of partially sterilised "tomato sick" soils.*

Soil *SB*. All extracts were made with ordinary distilled water, and food solution with water prepared in a silver still.

	Soil extracts				
			Partially sterilised soils		
	Food solution	Sick soil	Treated with toluene	Heated to 55°	Heated to 100°
Weight of produce—Root	·0257	·0215	·0205	·027	·0225
Shoot	·099	·046	·055	·058	·057
Total, grms.	·1247	·0675	·0755	·085	·0795
Length of root, cms.	30·2	31·1	31·5	29·0	32·1
Length of shoot, cms.	37·2	31·9	35·1	33·3	35·7
Experiment repeated later with the same soil—					
Weight of produce—Root	·0345	·030	·024	·030	·023
Shoot	·117	·063	·054	·070	·075
Total, grms.	·1515	·093	·078	·100	·098
Length of root, cms.	40·2	29·1	30·6	32·8	30·6
Length of shoot, cms.	37·1	29·2	29·0	33·9	33·8

were used and these made satisfactory growth; again, however, we could find no consistent differences between the plants in the extracts of the untreated, and of the partially sterilised soils. The composition of one of the extracts has been already given: some of the crop results are given in Table VI, while the plants are shown in Pl. V, fig. 4. A large number of other experiments have been made but the results were always negative.

Vegetation Experiments.

(*a*) *Cucumbers.* Having now shown that "sickness" is to be attributed to a low bacterial efficiency, and that it can be remedied under laboratory conditions by partial sterilisation, it remained to see if similar results could be obtained under the actual conditions of a commercial glasshouse, or whether other factors come into play to obscure these effects. Permission was therefore obtained from a large commercial grower, who has kindly taken very great interest in this work, to conduct an experiment in one of his cucumber houses. One section of the house was made up with old "cucumber sick" soil and another with the same soil heated by steam to 95–98° for four hours. One set of cucumbers was sown in boxes on Feb. 29th, and another set on March 4th, in both cases the plants on the sterilised soil came up later than those on the untreated soil and were at first inferior to them, but afterwards they went ahead and surpassed them. The plants were set out in the borders early in April and soon began to make rapid growth, those in the sterilised soils being much better than those on the sick soil. Yet the latter showed no signs of disease: their leaves were smaller and lighter in colour, but there was no club, wilt or any obvious insect or fungus pest. On May 6th fruit was cut from the plants on the sterilised soil, but none was ready on the plants on the unsterilised soil. The heating had, in fact, cured the "sickness" and rendered the soil commercially profitable once more.

Pl. III, fig. 2 shows a view of the house; typical plants were lifted and found to weigh:

	Green weight, grams	Dry weight, grams
On untreated sick soil	3025	158·7
On partially sterilised sick soil ...	3731	200·0

Samples of soil were taken from the sterilised and the untreated borders and were subjected to bacteriological and chemical examinations. Owing to the large proportion of dung present it is impossible

to obtain uniform and representative samples, but a general comparison can still be made:—

	Moisture per cent.	Bacterial numbers, millions per gram of dry soil		N per million of dry soil as	
				Nitrate	NH_3
	May 14	April 17	May 14	May 14	May 14
Untreated sick soil...	45	174	313	10·4	23·6
Heated sick soil	46·4	374	735	7·0	15·8

On both occasions the bacterial numbers are much higher in the heated than in the untreated soils. The amounts of nitrates and ammonia in both soils are low compared with the quantities recorded in Table II, an indication that absorption by the plant is well maintained, but it is rather more complete on the heated soil, as might be expected from the remarkable development of fibrous root.

(*b*) *Tomatoes.* A much more extended series of experiments was made with tomatoes since these plants do not require as high a temperature as cucumbers, and can conveniently be grown in an experimental house. Sick soil was obtained in sufficient quantity to enable us to carry the plants through to the end.

The seeds were sown in small pots (60's), the young plants were thinned out as soon as necessary and transplanted into larger pots (32's), then on to 10 inch pots (16's). During the growth of the plants certain qualitative differences were observed which are dealt with in another paper. The tomatoes were collected and weighed, and, at the end, the plants were lifted and weighed. Determinations were also made of the nitrogen in the plant, and of the nitrates left behind in the soil. It was thus possible to ascertain how far the soils in the greenhouse behaved like those in the laboratory experiments. The results are given in Table VII.

No strict comparison is possible between these values and those recorded in Table V, partly because of the difference in conditions and partly also because a certain amount of water always runs through the pot during the watering of the plants, and carries with it soluble nitrates. Moreover, in 1911 the plants were not re-potted sufficiently quickly and thus they received rather serious checks. In 1912 we had gained more experience of the crop and were able to remedy this defect, so that we consider these results altogether more reliable. In these

TABLE VII. *Dry matter and amounts of nitrogen in tomato plants grown in untreated and partially sterilised "tomato sick" soils.*

1911 results. Dry matter obtained in root, shoot and fruit, grams.

	Soil *MT*	Soil *MC*	Soil *SC*	Cucumber sick soil *B*
Untreated soil	66·88	30·50	58·31	40·04
Soil heated to 98°	103·50	62·49	80·00	75·27
" " + basic slag	—	67·89	—	78·76
" " 55°	64·77	37·24*	57·40	45·00
Soil treated with 0·5% toluene	77·26	44·55	—	51·22
" " " CS_2	76·65	44·29	—	46·80
Nitrogen in root, shoot and fruit, grams.				
Untreated soil	0·947	0·437	0·835	0·645
Soil heated to 98°	1·484	0·879	1·313	1·168
" " + basic slag	—	0·943	—	1·328
" " 55°	·866	0·594	0·891	·606
Soil treated with toluene	1·186	0·678	—	·703
" " CS_2	1·251	0·628	—	·758

* In this case the temperature of heating was 45° only.

1912 results. (Plants cut early.)

	Dry matter in shoot and root, grams	N in root and shoot, grams	N left as nitrate in soil, grams	Total N converted into nitrate and NH_3	
				grams	parts per million of dry soil
Untreated soil	7·1	·207	·098	·340	58
Soil heated to 98°	26·8	·674	·085	·789	131
Soil treated with formaldehyde	16·6	·369	·068	·465	82
" " pyridene	13·2	·419	·301	·756	126
" " CaS	13·2	·352	053	·435	72
" " petrol	12·1	·298	·057	·402	68
" " toluene	11·8	·295	·101	·431	74
" " phenol	9·0	·218	·091	·339	58

experiments the amounts of nitrogen obtained by the plant from the partially sterilised soils correspond fairly closely with the laboratory results, an indication that no notable disturbing factor comes into play in the pots.

In at least two cases, however, a serious discrepancy was observed between the action of toluene under laboratory conditions and its behaviour in the pot experiments; the increased rate of decomposition

was induced in the laboratory but not in the pots. The figures for these two soils are:

	Soil *MT*		Soil *A*	
	Ammonia and nitrate produced		Ammonia and nitrate produced	
	In Laboratory expts. (Table V)	In pots	In Laboratory expts.	In pots
Untreated soil ...	100	100	100	100
Toluened soil.....	143	104	125	99

The difference was traced to the low solubility of the toluene vapour. In the laboratory experiments the soil is sifted very finely and is confined in small quantities (800 grams) in closed bottles for 36–48 hours while the toluene acts. In the pot experiments this fine sifting is impracticable because it would lead to serious panning; coarse lumps are always present, and the treatment with toluene is carried out in large pots so that the extent of penetration is much reduced. Chemical analyses of soils treated in this way showed that nitrification was checked and ammonia accumulated, but there was no increase in the rate of decomposition. Two soils were found to contain in parts per million:

	Soil *RC*, 30 % moisture			Soil *A*, 32 % moisture		
	Ammonia, after 62 days	Ammonia and nitrate, after 62 days	Relative quantities	Ammonia, after 84 days	Ammonia and nitrate, after 84 days	Relative quantities
Untreated...	7	156	100	9	312	100
Imperfectly toluened	71	165	106	74	306	98

Our experiments show that toluene acts best on finely sifted fairly dry soils, and loses much of its effectiveness in rich soils when too much moisture is present, or the particles are too coarse.

The population of the untreated and partially sterilised "sick soils."

Examination of the sick soils at various times has shown the presence of the following groups of organisms (p. 108):

	Untreated soil	Soil heated to 98° C.	Soil heated to 55° C.	Treated with toluene	Treated with carbon disulphide
Fauna	Protozoa (various)*	None	—	Certain flagellates present, others killed	Certain flagellates present, others killed
	Rotifers	,,	None	—	—
	Eelworms—Heterodera	,,	,,	Considerably reduced	Considerably reduced
	Free living forms	Killed but were reintroduced later	Killed but were reintroduced later	Rarely present but reintroduced later	Killed but were reintroduced later
	Enchytraeid worms	None	None	Present	Present
	Earthworms	,,	,,	—	—
	Woodlice	,,	,,	Some killed, others driven away	None
	Millipedes—	,,	,,	Present	—
	Julus terrestris				
	,, *pulchrellus*				
	Polydesmus complanatus				
	Centipedes	,,	,,	—	—
	Springtails	Introduced later	Introduced later	Introduced later	Introduced later
	Mycetophylid larvae	None	None	—	—
	Wireworms	,,	,,	Present	None
Flora	*Pythium de Baryanum*	,,	Rare	As bad as in untreated	As bad as in untreated
	Plants readily damped off	No damping off. Much *Pyronema glaucum* (Bondier)†	Occasional damping off	—	—
	Fusarium sp.	—	—	Fusarium sp.	Fusarium sp.

* Some of these have been described by Mr T. Goodey in *Proc. Roy. Soc.* 1911, **84** B, 165—180 and by Mr. C. H. Martin in *Proc. Roy. Soc.* 1912, **85** B, 393—400.

† Kindly identified for us by Mr Carleton Rea.

The plants on the "sick" soil are liable to damp off in early life and to be attacked by *Heterodera radicicola*, but "sickness" is not conditioned by these circumstances. Cases have come within our knowledge where plants on sick soil escaped these pests and yet showed the characteristic debilitated state. Our experimental tomatoes suffered from no obvious fungoid disease, probably through the mere accident that the house is well isolated, for the plants on the sick soil looked as if they would take any disease that happened to be about. This weakened physiological condition of the plants on the sick soil no doubt accounts for the close connection between soil sickness and incidence of disease.

The commercial treatment of sick soils.

The present method of dealing with cucumber sick soil is to throw it away at the end of the season, or else to carry it back to the field, sow it down with grass, and leave it alone for a few years, then once more bring it back to the cucumber house. This method is so wasteful that any alternative deserves consideration. Our experiments have shown that partial sterilisation affords a satisfactory method of treatment, and trials made under our observation in commercial glasshouses show that it is also practicable on the large scale.

Two general systems may be adopted: the soil may be heated to 90–100° C. or it may be treated with some antiseptic. These systems are fundamentally different. At 90–100° a certain amount of decomposition takes place with formation of products having important secondary effects on the plants which will be discussed in a later paper. Treatment of the soil with antiseptics causes much less decomposition, but certain secondary effects are seen in this case also. Thus in discussing methods of treatment capable of application on the large scale regard must be had not only to the cost and practicability, but also to

(1) the effect on the bacterial activity in the soil,
(2) the effect on disease and parasitic organisms,
(3) the secondary effect on the plant.

Of all methods we have tested so far heat is much the best, but the present cost of heating one ton of soil is about 1*s.* to 1*s.* 6*d.* At this price the process is applicable for growing cucumbers, tomatoes in pots, and certain other glasshouse crops. Chemical treatment is much cheaper and promises greater possibilities of development. Any antiseptic may be expected to serve, but there are two conditions that

must be fulfilled in practice: (1) the antiseptic must be sufficiently soluble to get about in the soil and penetrate the particles, (2) either it must be volatile or it must decompose with formation of innocuous compounds after its work is done, so that it shall not exert any permanent harmful effect on the plant or the food-making bacteria. A large number of substances fulfil these conditions, and the question of selection resolves itself into a comparison of the cost and the effectiveness in the three directions above mentioned. Toluene, which we have used in our laboratory experiments, is unsuitable for commercial work. Apart from the difficulty of transport it is not sufficiently soluble to penetrate the soil particles if much moisture or organic matter is present. Formaldehyde gives very good results and has the great advantage of solubility. Certain tar oils are also very promising, and these when insoluble can be made miscible with water by bringing the specific gravity to 1. In dealing with any commercial product, however, it is always necessary to know the behaviour of the separate constituents in the pure state. An illustration was afforded by commercial toluene. Our most recent pot experiments with pure toluene have given us less satisfactory results than the earlier experiments; other cases have also come to our notice where toluene has behaved in rather a varying manner, sometimes giving fairly large increases and sometimes not. Dr Hodgkinson suggested to us that the sulphur compounds commonly present in toluene (thiophen and thiotolen) were probably the cause of the irregularities; until recently even the purest toluene sold by the dealers contained these sulphur compounds, but lately the specifications of the chief purchasers have been altered so as to require their removal. Thiophen is unfortunately very costly, but Dr Hodgkinson kindly gave us a large supply that he had prepared himself, and we tried its effect on the soil. It proved to be much more active than pure toluene, as shown in Pl. V, fig. 5; indeed pure toluene did not produce any marked effect in this experiment, probably because of its low solubility.

We have had a similar experience with one of the bye-products obtained during the isolation of naphthalene from tar. This particular oil proved to be distinctly useful, and we accordingly fractionated it to get out its chief constituent in a tolerably pure state. But this purer substance was much less useful than the crude oil, and it was clear that the most potent constituent was present in relatively small amounts.

Examination of various waste products and their pure constituents

is in hand. In the meantime we cannot too strongly insist that no waste product should be recommended as a means of treating soil sickness until it can be obtained to a definite specification, and the behaviour of its separate constituents is known.

Conclusions.

1. Sickness in glasshouse soils is conditioned by at least two factors:

(*a*) an accumulation of insect and fungoid pests,

(*b*) a lowered bacterial efficiency.

2. The lowering of the bacterial efficiency is due to the accumulation of a factor detrimental to bacteria.

3. The sick soils examined did not appear to contain any substance toxic to plants or bacteria. The soils were well supplied with plant food and with calcium carbonate.

4. The factor detrimental to bacteria resembles in every way that present in ordinary arable soil. It is put out of action by heat or by antiseptics. It is not associated with the bacteria but with the soil, and is capable of growth when introduced into partially sterilised soil. In all respects its properties agree with those of protozoa.

5. There is no evidence that sickness is due to an accumulation of bacteria acting unfavourably on the production of plant food (*e.g.* denitrifying bacteria) or that the beneficial effect of partial sterilisation is due to the destruction of such bacteria. So far as we can find all bacterial actions are accelerated in partially sterilised soils; there is, for example, a marked increase in the rate of loss of nitrogen.

6. Soil sickness in tomato and cucumber houses can be effectually treated by partial sterilisation.

Fig. 1. Tomato plants grown in untreated and in partially sterilised tomato sick soils.

The treatment was effected by adding antiseptic equal to 0·25 % of the weight of the soil (except in the case of formaldehyde when 0·1 % was used), leaving it in the soil for 2 days and then allowing it to evaporate before the soil was used. The heating was effected by steam and was continued for 3 hours.

Heated Untreated

Fig. 2. Cucumbers grown in partially sterilised and in untreated cucumber sick soil.
The partial sterilisation was effected by steam heat continued for 3 hours.

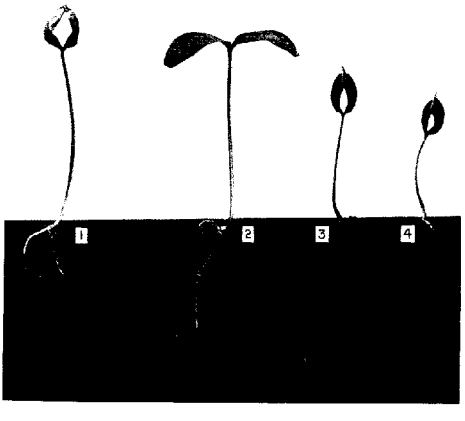

Fig. 3 *a*. Cucumbers grown in water cultures. 5 days' growth.

(1) Normal food solution.
(2) Water extract of cucumber sick soil.
(3) Water extract of cucumber sick soil made after the soil had been heated for 3 hours to 98° C. by means of steam.
(4) Extract made after the sick soil had been treated with 0·5 % of toluene.

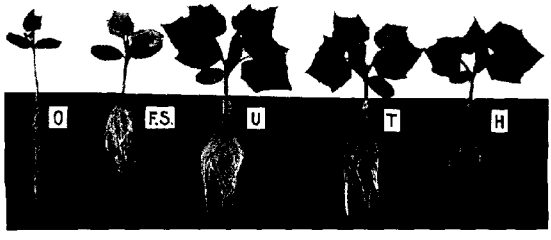

Fig. 3 *b*. Cucumbers grown in water cultures. 5 weeks' growth.

O. Tap water alone. F.S. Normal food solution in tap water.
U. Extract of cucumber sick soil in tap water.
T. Extract made with tap water after the soil had been treated with 0·5 % of toluene.
H. Extract made with tap water after the soil had been heated for 3 hours to 98° C. by means of steam.

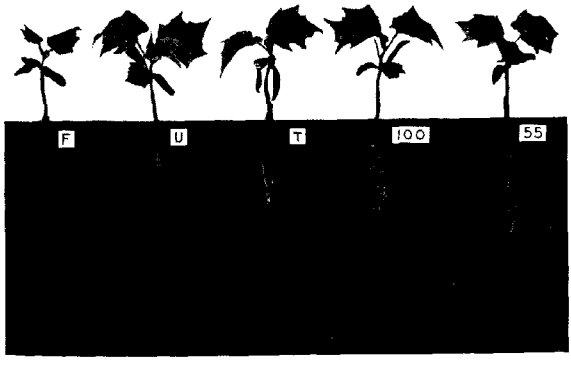

Fig. 3 c. Cucumbers grown in water cultures. 6 weeks' growth.

F. Normal food solution in ordinary distilled water.
U. Extract of cucumber sick soil in ordinary distilled water.
T. Extract made after the soil had been treated with 0·5 % of toluene.
100. Extract made after the soil had been heated for 3 hours to 98° C. by means of steam.
55. Extract made after the soil had been heated for 3 hours to 55° C.

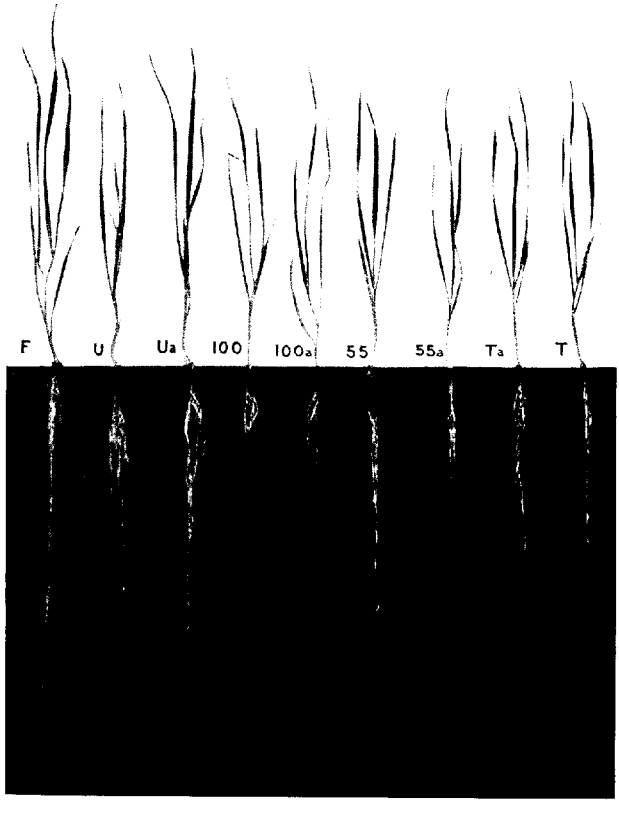

Fig. 4. Barley grown in water cultures.

F. Normal food solution. The others are extracts of tomato sick soil, untreated (U) or partially sterilised (100, 55, T: these having the same meaning as in Fig. 3c). The letter *a* indicates that the extract has been boiled for ½ hour, a treatment which is seen to have little or no effect in this experiment.

Thiophen Toluene Untreated

Fig. 5. Young tomatoes growing in tomato sick soil.

Pure toluene has had but little effect in this experiment but thiophen is much more potent.